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Abstracts

European Society of Urological Oncology and Endocrinology 5th Congress, 18th–20th August 1986, Edinburgh, UK

The ESUOE began in 1979 in Amsterdam as the result of the efforts by a small group of biochemists and urologists to link their mutual interests in steroid receptors and metabolism, especially of prostatic cancer. The Society has flourished and has had further meetings in Stockholm, Rome, Amsterdam and now looks forward to its 5th Congress, to be held in Edinburgh in August 1986.

The following abstracts indicate that the subject material of the Society Congress has expanded into a wide range of topics where both biochemists and urologists continue to have strong mutual interests. The links between the laboratory and the bedside are often difficult to establish but we believe that this is an essential role for this Society so that much of our work can be directed at the many oncological problems that still resist our treatments.

Geoffrey D. Chisholm, Chairman, 5th Congress ESUOE

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EPIDERMAL GROWTH FACTOR RECEPTORS (EGFr) IN BLADDER CANCER. A.L. Harris*+, K. Smith+, D. Neal°, R.R. Hall°. Tumour growth factors can stimulate cell division by binding to the EGFr on surface membranes and stimulating intracellular tyrosine kinase activity. We decided to see whether EGFr could be detected in human bladder cancer and how they correlated with biological behaviour. We studied 48 patients with bladder cancer; 40 male, 8 female. The median age was 62 years (range 35-90). 24 patients had superficial transitional cell carcinoma (15 pTa, 9pT1); 5 were poorly differentiated (2 pTa, 3pT1) and 19 moderately differentiated. 24 patients had invasive transitional cell carcinoma (pT3); 16 were poorly differentiated and 8 moderately differentiated. The EGF receptor was identified by means of an indirect immunoperoxidase technique with a murine monoclonal antibody (EGFR1; donated by Dr M. Waterfield). 7 of the 24 superficial tumours (29%) were graded positive for EGF receptors. 21 of the 24 invasive tumours (87.5%) were positively stained for EGF receptors. Thus the proportion of patients positive for EGF receptors was significantly greater for those with invasive than for those with superficial tumours ($X^2 = 14.49$; $p < 0.001$). Significantly more of the poorly differentiated tumours (18 of 21) than the moderately differentiated tumours (10 of 27) were positively stained $X^2 = 9.6$; $p < 0.01$). Membranes were prepared from the tumours and EGFr quantitated by ligand binding using ^{125}I labelled EGF. There are 2 classes of receptor, one with high affinity $\simeq 10^{-9}$M. On some invasive tumours there was 10X more EGFr than on superficial tumours, suggesting the possibility of gene amplification. Tumour growth factors produced locally may stimulate more aggressive behaviour in these tumours. The long-term implication of expression in superficial tumours is under study currently. The EGFr provides a target for selective therapy and we are using monoclonal antibodies to EGFr for targetting in human bladder cancer.

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EXPRESSION OF ALPHA- AND BETA-TRANSFORMING GROWTH FACTORS IN A HUMAN BLADDER CANCER CELL LINE.

W. Heckl and H.-W. Vohr.

Normally occurring as well as pathologic growth-promoting substances have been implicated in neoplastic transformation and growth. Transforming growth factors (TGF) and the epidermal growth factor (EGF) which are excreted in the urine, are particularly relevant for studies of bladder carcinoma, since EGF is a potent mitogen with co-carcinogenic properties that have been associated with malignant, pre-malignant and normal urothelium.

The acidic-acid-ethanol extracts of the human bladder carcinoma cell line EJ, which is known to contain the ras-oncogene were examined for the presence of transforming growth activities.

Fractions of the cell extracts on Bio-Gel-P30 revealed the presence of growth stimulating factors within the range of M.W. 6.000 and 29.000 using NRK-49F-cells as indicators.

The colony forming activity of the heat and acid resistant and trypsin and dithiothreitol sensitive proteins was 4-fold enhanced upon addition of EGF. The collected fractions with the highest stimulating activity were further purified by reverse-phase HPLC using a Baker C-18 column. The majority of the transforming growth activity eluted at 38% acetonitrile. These results indicate that the human bladder cancer cell line EJ contains alpha- and beta-TGFs, which in part might be responsible for the tumour cell proliferation of the bladder tumour.

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BIOCHEMICAL AND IMMUNOLOGICAL EVIDENCE FOR THE PRESENCE OF EGF RECEPTORS IN BPH. S.Q. Maddy, G.D. Chisholm, R.A. Hawkins* and F.K. Habib.

The aetiology and pathogenesis of BPH are poorly understood. Epidermal growth factor (EGF) have been shown to exert pronounced hyperplastic effects on epidermal tissues. These effects are thought to be mediated by the interaction of the peptide with a specific plasma membrane receptor protein. The uptake of ^{125}I labelled mouse epidermal growth factor by human hyperplastic prostate was investigated. Specific binding was found to be present in about 80-90% of tissues. The binding was both time and temperature dependent, with maximum specific uptake achieved after incubation for 60 min at 37°C. Scatchard plot analysis of ^{125}I binding revealed two binding sites for EGF with K_d values of 7.5 and 16 nM. Subcellular fractionation indicated that the bulk of the specific EGF binding was confined to the 800g pellet with a little binding in the cytosol fractions. Heating the homogenate at 45°C for 10 min prior to incubation completely abolished the binding. Also pre-treating the homogenate with trypsin at 37° for 90 min produced the same effect. Unlike our earlier studies with prolactin we found that pretreatment with charcoal and $MgCl_2$ reduced the specific binding. Insulin, prolactin, FSH and LH did not compete with the EGF for binding. In another experiment indirect immuno peroxidase staining revealed the presence of binding sites for EGF on cut sections of BPH tissue. These studies therefore demonstrate that the prostate is a potential target tissue for EGF.

This study was supported by a grant from the World Health Organisation.

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FREQUENCY OF RAS GENE ACTIVATION IN HUMAN BLADDER CARCINOMA.

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Activation of ras gene has been reported in many different human cancers where three of the five members of the ras gene family (c-Ha-ras-1, c-Ki-ras-2, n-ras) have been detected using NIH/3T3 transfection assay. One study has reported the frequency of molecular alteration affecting ras proto/oncogenes in human urinary tract tumours where 3 of 38 tumours DNA's studied (less than 10%) revealed point mutations associated with activation of 1 member of the ras gene family, c-Ha-ras (Proc. Natl. Acad. Sci, USA (1985), 82, 3849). Using an alternative transfection assay with tumorogenic potential as an end point, we have surveyed 25 human bladder carcinomas and identified activated ras gene in at least 5 of these samples (20%). Three have been identified as c-Ha-ras-1. Our studies reveal a higher incidence of ras activation in bladder carcinomas than previously reported but demonstrate no strong correlation between this and any particular tumour pathology. The presence of c-Ha-ras-1 is suggestive of a preferential activation of this ras gene member in urothelial tumours. Molecular analysis of the changes responsible for ras gene activation in these tumours is in progress.

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RAS ONCOGENES AND BASAL LAMINA PROTEINS IN BLADDER CARCINOGENESIS. P.R. Malone, M. Warburton, B.A.J. Ponder R. J. Shearer, I.C. Summerhayes

Recent studies implicating the c-Ha-ras-1 oncogene in the causation of bladder cancer have judged the malignant potential of this gene by its effect on a murine fibroblast cell line (NIH/3T3). To give this work more direct relevance to bladder carcinogenesis we have studied its action on non-transformed murine transitional epithelial cells (BBN3 and BBN7). The cloned gene from the EJ bladder carcinoma cell line was introduced into these cells by calcium phosphate DNA transfection. In view of recent interest in the role of basal lamina defects in carcinogenesis we have looked at the organisation of Type 4 collagen, laminin and fibronectin in parental and transfected cell lines by immunofluorescence and immunoprecipitation.
Transfected cells became tumorigenic and grew in soft agar. They also showed altered handling of Type 4 collagen and laminin showing an inability to form an extracellular matrix and developing intracellular inclusion bodies, although these proteins were detectable in the culture medium by immunoprecipitation. The organisation of fibronectin was unchanged.
The observation that ras gene activation can interfere with basal lamina protein organisation is an interesting finding and warrants further investigation.

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CELL CULTURE AS A TOOL FOR PROSTATIC CANCER RESEARCH. F.H. Schröder

Interest in cell culture as a research tool in prostatic tumors started at least as early as 1917, when Burrows, Burns and Suzuki published their report on "The Cultivation of Bladder and Prostatic Tumors outside the Body" as the first paper in the first issue of the Journal of Urology. They observed outgrowth of epithelial cells from prostatic explants on plasma clots which they were able to maintain for about 24 hours. In the meantime, many advances have been made: synthetic media and plastic dishes are available, subculturing is easily possible, tissue preparation allows direct plating of cell suspensions and cloning is possible in an automated fashion. Still, progress of our knowledge of the behaviour of prostatic cells in vitro has been limited. It has been difficult to obtain growth of all desired cell types. Recent advances reported by Pehl and Stamey by using cholera toxin and other medium supplements have not been reproducible by others. Characterization of cells as normal cells, cancer cells, stroma elements, etc. has been difficult to the degree that it still remains doubtful whether cancer cells can reproducibly be grown in vitro. The development of long term lines turned out to be extremely difficult. Once a number of such lines were developed, they were shown not to reflect very well the properties of prostatic cell populations that were to be studied. Still, some approaches, mainly those related to short term cultures, the application of new media and co-cultivation appear useful and should be pursued.

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DEVELOPMENT OF AN IN VIVO CLONOGENIC CELL ASSAY FOR RAT PROSTATE METASTATIC TUMOR - R3327-MatLyLu. A.A. Geldof, B.R.Rao and H.J. de Voogt.

Cancer treatment should be directed towards the eradication of the proliferating tumor cells, responsible for tumor growth and metastasis. Next to in vitro clonogenic cell assays, effects of agents on tumor cells should be evaluated under in vivo conditions. In order to develop a lung colony assay we studied the organ distribution of radiolabelled (^{3}H-Thymidine) R3327-MatLyLu rat prostate tumor cells after intravenous (iv) injection. Injected cells entered the lungs rapidly in a high proportion (±70% at 30 min.) in comparison to kidney, spleen, liver, testis, intestine, prostate and muscle. At 24 hours only 2% was present in the lungs. Radiolabelled non-tumorigenic cells used as control entered the lungs not in such a high proportion as did the tumor cells. Ten days after iv injection of non-radiolabelled tumor cells, macroscopically recognisable pleural colonies, histologically similar to the primary tumor, were observed. A linear relationship between the number of cells injected and the number of lung colonies observed was established. Recognisable colonies were not observed in any other organ examined.
The effect of in vitro treatment of a tumor cell suspension with cisplatinum (1 hour,37°C,5µg/ml) was analyzed using both lung colony and in vitro agar colony assays. The surviving fraction measured was lower using the lung colony assay (0.40 ± 0.08) compared to the in vitro agar assay (0.63 ± 0.02).
Conclusion: Information obtained from in vitro can be supplemented with that from in vivo assays, providing a better understanding of chemotherapy effects on tumor cells.
Thanks to M.Meulenbroek, P.Jeucken and H.Valster for technical assistance and to Netherlands Cancer Foundation, Maurits en Anna de Kok Stichting and Vrije Universiteit for research support.

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HORMONAL MODULATION OF THE GROWTH OF A NEW TRANSPLANTABLE PROSTATIC CELL LINE IN ATHYMIC NUDE MICE. M.E. Harper, P.E.C. Sibley, A. Rowlands, L. Buttifant, C. Beacock and K. Griffiths.

Multiple models in vitro and in vivo of human prostatic cancer are required for the development and preliminary testing of potential agents for the treatment of this disease. A few human prostatic tumour cell lines have been established in cell culture or as xenografts in athymic nude mice, mainly from metastatic prostate tissue. A new human prostatic cell line, TEN/12, from a primary tumour, has been serially passaged in athymic nude mice which should prove to be a useful additional model. The tumour displays both anaplastic and glandular regions, pleomorphic nuclei and abundant mitoses (65/1000 cells). Heterogenous distribution of prostatic acid phosphatase and prostate specific antigen expression is seen in sections of the xenografts. Extensive vascularization of the primary tumour and xenografts has prompted the search for angiogenesis factor expression in these cells.

The TEN/12 xenografts appear to be androgen sensitive as excellent growth is observed in intact males and testosterone supplemented males but no tumours have yet been observed in females or castrated males. Analysis of nuclear steroid hormone receptor indicated high affinity binding with a K_d 4.1 x 10^{-9} M/l and a receptor content of 611 fmoles/mg DNA. Various hormonal modalities are presently being tested using this in vivo model. The cells from the xenografts can be cultured in semi-solid agar containing medium DMEM, supplemented with 10% foetal calf serum, insulin and 5α-dihydrotestosterone, so providing an in vitro experimental model of prostatic carcinoma.

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COLONY GROWTH DYNAMCIS IN VITRO AND CELL VIABILITY OF THE MATLYLU TUMOUR AND SIX DUNNING RAT PROSTATIC TUMOUR CELL LINES.

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Recently, six different Dunning Rat Prostatic Tumour Cell Lines were established by in vitro cultures. In this study we have investigated the growth potential of the MATLyLu tumour and six different Dunning rat prostatic tumour cell lines in the human tumour cloning system (HTCS). Flow cytometric analysis of tumour cells from the solid MATLyLu tumour before and after culture showed that in HTCS only the cell population with an abnormal DNA stemline gave the formation of colonies. The evaluation of the growth pattern in vitro with growth curves and an automated colony counter showed that there was no essential difference in routine counting procedures of colony formation in comparison to the use of a vital cell staining method. The use of a cytotoxic agent (mercuric-chloride), applied in an overlayer technique after cell plating, did not allow colony formation but it was not possible with this method to destruct colonies totally once they were present in culture. All six different tumour model systems showed the formation of colonies in HTCS with a non-linearity of cell growth.

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ANDROGENS IN A TRANSPLANTABLE HUMAN PROSTATE CANCER LINE (PC-82): CORRELATION WITH PROLIFERATION.

G.J. van Steenbrugge, J.J.W. van Dongen, F.H. de Jong, M.P.W. Gallee and F.H. Schroeder

The hormone-dependent human prostatic tumor line, PC-82, which is permanently transplantable in nude mice, was used as a model to study the effects of hormonal manipulation on tissue levels of androgens and on the proliferative activity of the tumor. Endogenous levels of testosterone (T) and 5α-dihydrotestosterone (DHT) were measured in whole tissue homogenates by radioimmunoassay after separation on silica columns. The proliferative activity of the tumor tissue was estimated in frozen tissue sections using a monoclonal antibody, Ki-67, directed against a cell proliferation-associated nuclear antigen (PaNA).
In PC-82 tumor tissue grown in intact male mice mean levels of T and DHT were 22 and 18 pmol/g tissue respectively. Tissue grown in mice receiving a Silastic T-implant (providing constant plasma levels of the hormone) contained similar levels of T and DHT, with a smaller inter-individual variation than found in intact mice. Androgen withdrawal in PC-82 bearing T-implanted female mice resulted in a decline of the concentration of T and in a more gradual decrease of DHT in the tumor tissue. After 10 days T and DHT concentrations were below 0.5 pmol/g tissue and the expression of the PaNA had decreased from about 20% in control tissue to 0.2% in tissue grown in mice depleted of androgen. In these androgen depleted tissues control levels of T and DHT were restored within two days after reimplantation of T. This androgen repletion resulted in a rise of the tumor cell proliferative fraction to 20% within four days. The present results with the PC-82 tumor model yield valuable information on the effects of hormonal manipulation on steroid levels in human prostatic carcinoma tissue. The Ki-67 antibody provides a reliable method for estimation of the proliferative fraction of hormone-responsive cancer tissue.

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UROLOGIC CANCER CELL LINES: DIFFERENTIAL SENSITIVITIES TO CHEMOTHERAPEUTIC DRUGS AND RADIATION.

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Advanced testicular germ cell tumours can be cured using chemotherapy, in contrast to most other types of urologic cancer. In order to determine whether this differential sensitivity is retained in vitro, we compared the response to cisplatin, adriamycin and γ-irradiation of human continuous cell lines derived from five non-seminomatous testicular germ cell tumours and five transitional cell carcinomas of the bladder. The range of drug concentrations required to reduce clonogenic cell survival by 70% were:

	TESTIS	BLADDER
ADRIAMYCIN (ng/ml)	0.9 - 7.7	3.7 - 22
CISPLATIN (ng/ml)	20 - 170	112 - 420

Thus, the cell lines retain their relative chemosensitivities in the absence of humoral factors, indicating that testicular tumour cells are inherently more sensitive to these drugs, and that response is not dictated indirectly by factors such as blood supply and immunogenicity. Similarly, the testicular cell lines were all more sensitive to γ-irradiation than the bladder cell lines, with dose-response curves similar to those of patients with ataxia telangiectasia, a disease associated with defective DNA repair.

It is concluded that these cell lines provide a model system for studying the molecular basis of drug sensitivity, a line of investigation which could lead to more effective therapy for all tumours.

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MULTICELLULAR BLADDER TUMOR SPHEROIDS IN CO-CULTURE WITH HUMAN ENDOTHELIAL CELL MONOLAYERS

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The adherence of tumor cells to the vascular endothelium and interaction is an important step in the pathogenesis of metastasis. To study the interaction between tumor cell aggregates and endothelial cells in vitro, we used multi-cellular-tumor-spheroids (MCTS), of the human bladder carcinoma cell line J 82 and monolayer cultures of human endothelial cells from umbilical veins grown on bovine basement membranes. MCTS in different growth phases were placed on the endothelial cell monolayers and analyzed after coculture periods of 1 hr, 6 hrs and 1, 2, 6 and 10 days. Morphological changes both of MCTS and endothelial cells were studied by TEM and SEM. Cell proliferation was evaluated using the BrdU-anti-BrdU technique.
Whereas the growth of MCTS is not influenced by the cocultured endothelial cells, endothelial cells, after initial degeneration and necrosis, show a high rate of proliferation around MCTS, when compared with control particles. The interaction of tumor cells within the MCTS itself differs from that of outgrowing tumor cells.
The model system used allows to study the basic cellular interactions between tumor cell aggregates and endothelial cells in situ, independent from general influences of immunological or coagulatory mechanisms.

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ANDROGEN RECEPTOR STATUS IN CANCER OF THE PROSTATE: THE IMPACT OF THE STAGE AND GRADE OF THE TUMOUR ON RECEPTOR CONTENT.

F.K. Habib, S. Odoma, A. Busuttil and G.D. Chisholm.

Nuclear and cytosolic androgen receptor concentrations were measured in 13 patients with cancer of the prostate (CaP) and these values were compared to those in an age matched group of 23 patients with benign prostatic hyperplasia (BPH); None of the cancer patients had received any therapy before entering into this study. Prior to transurethral resection, all malignant tumours were clinically staged by digital palpation. Histological grading was carried out by a single pathologist according to the Gleason system. The mean ± S.E.M. receptor values for BPH (cytosol: 115 ± 18 fmol/g tissue; nucleus: 140 ± 34 fmol/g tissue) were not significantly different from those measured in CaP (cytosol: 105 ± 23 fmol/g tissue; nucleus: 83 ± 23 fmol/g tissue). The results show an overlap between the two groups and demonstrate the inability of cytoplasmic and nuclear androgen receptors to differentiate between CaP and BPH. Our data also revealed the absence of any correlation between histological grade in CaP and receptor content. If however the tumours were classified according to the stage of the cancer using the TNM system, 'early disease' tumours maintained significantly lower Gleason Score (4.4 ± 0.61) and receptor levels (cytosol: 63.8 ± 31.2 fmol/g tissue; nucleus: 46.2 ± 26.5 fmol/g tissue) than those measured in the 'late disease' (Gleason Score: 7.0 ± 0.56; cytosol receptor: 146.2 ± 20.5; nuclear receptor: 117.2 ± 31.6) ($p < 0.05$). Although the staging of the disease bears a great impact on the capacity of the tumour to specifically bind androgens, it is still uncertain whether androgen receptors alone will be sufficient to provide a useful predictive index of response to endorcine therapy.

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DOES THE NUCLEAR ANDROGEN RECEPTOR CONTENT FROM CANCEROUS PROSTATIC TISSUE PREDICT THE DURATION OF RESPONSE FOLLOWING ORCHIECTOMY IN PATIENTS WITH METASTATIC DISEASE OF THE PROSTATE? O. van Aubel, J. Bolt-de Vries, M. Blankenstein and F. Schröder.

The nuclear androgen receptor (ARn) content of prostatic tissue has been investigated as a means of predicting the response of prostatic carcinoma to endocrine therapy. Trachtenberg and Walsh (J.Urol., **127**, 466, 1982) and Ghanadian et al (Lancet, **26**, 1418, 1981) found that the ARn content was related to duration of response and survival following hormonal therapy in advanced prostatic cancer. In 1981 a prospective study was started to investigate whether the ARn content in biopsy specimens of patients with prostatic carcinoma predicts the duration of response following hormonal treatment. ARn was estimated by a micro assay, which involves extraction of nuclear pellets with a heparin containing buffer, exchange labelling of the nuclear extract with ^{3}H-R1881 and quantitation of the receptor with protamine sulphate precipitation (J. Foekens, Clin. Chim, Acta, **109**, 91, 1981). 115 patients with prostatic cancer entered the study, 45 patients had evidence of metastatic disease to bone proved by bone scan. 44 patients were treated by orchiectomy, 33 of these patients are evaluable with a follow up of 22-41 months, 24 patients had progression after 2-30 months, 1 patient had no response, 8 patients with a follow up of 22-41 months have stable disease. Until now we could not find a correlation between the nuclear androgen receptor content and the duration of response following orchiectomy in these patients with metastatic disease of the prostate.

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PUNCH BIOPSY TISSUE: ARE THERE LIMITATIONS TO ENZYME AND RECEPTOR ANALYSES. G. Bartsch, O. Dietze and G. Mikuz

Significant quantitative differences have been demonstrated between normal prostatic and carcinomatous tissue in regard to androgen binding capacity, metabolism and endogenous androgen concentrations.

A morphometric analysis of the biopsy specimens of 50 patients with prostatic carcinoma was performed. This analysis showed cancer cells in 23% of the specimens analysed and stromal tissue in 72%. Normal or BPH-changed glandular cells were observed in 4% of all biopsy specimens.

The stromal tissue of the elderly male is highly activated and sensitive to androgens and estrogens. In a correlative biochemical and morphometric study on the androgen metabolism in the prostate the 5 α-reductase activity of the stromal part was found to occur in a similar degree as in the glandular part.

Consequently enzyme and receptor assays of biopsy specimens have to be interpreted very cautiously, since androgen metabolism and binding may result not only from cancer cells but also from the stromal tissue, which is the case in a high percentage of patients. Therefore these biochemical assays should be confirmed by quantitative morphology of the tissue distribution in future; otherwise the prognostic value of these endocrinologic investigations might be compromised.

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ANDROGEN-RECEPTOR DISTRIBUTION IN HUMAN PROSTATE NUCLEI. P. Davies and N. Kyprianou

Both prostate cancer and benign prostatic hypertrophy (bph) are conditions in which nuclear androgen receptors may differ quantitatively or in intranuclear deployment from the normal conditions. Such anomalies may be related to the aetiology of the disease, and comparisons between the two may yield information on the disparate development of the two conditions. In this study, nuclear androgen receptors were compartmentalized on the basis of release during nucleolytic disruption by micrococcal nuclease. Thus, receptors can be defined as free from association with nuclear structures, chromatin-bound, or matrix-bound. The large variability in nuclear androgen-receptor content of bph epithelial cells was due to a numerically variable population of uncommitted receptors i.e. not necessarily associated with either chromatin or matrix. However, in in vitro reconstituted systems, these receptors were capable of binding to DNA, chromatin and matrices. In contrast, prostate cancer nuclei were characterized by a predominance of nuclease-resistant receptors. This was not a function of total nuclear-receptor content. Therefore, bph may result from a superabundance of receptors available to hyperstimulate biochemical processes, whereas prostate cancer may reflect the availability of anomalous acceptor sites directing receptor activation into new and potentially maleficial pathways.

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HISTOCHEMICAL STUDY OF ANDROPHILIC PROTEIN IN HUMAN PROSTATIC CANCER

H.SUMIYA, K.YAMAGUCHI, H.FUSE, H.ITO, O.MATSUZAKI and J.SHIMAZAKI

Since it has been discussed that prostatic cancer with positive androgen receptor shows better prognosis, this study was undertaken to observe relationship between presence of androphilic protein and responce to endocrine therapy in prostatic cancer. Previously we reported histochemical detection of androphilic protein in the prostate with R1881-CMO-BSA-FITC (a conjugate of fluorescein isothiocyanate and bovine serum albumin bound with R1881-3-carboxymethyloxime)(Invest.Urol. 18:337,1981).

1)Fluorescence of DHT(dihydrotestosterone) and mibolerone (7α,17α-dimethyl-19-nortestosterone) were compared with that of R1881 (methyltrienolone) and it was revealed that R1881 and mibolerone were better ligand than DHT. Thus routine examination was performed with R1881.

2)Preincubation with 1μM of triamcinolone acetonide diminished fluorescence.Together with other inhibition study, it was suggested that fluorescence studied with R1881 were sum of androgen receptor and progestin-binding protein.

3)Stage D_2 prostatic cancer was examined on androphilic proteins with R1881(62 cases) and DHT(54 cases).The responsiveness to the endocrine therapy was judged at 6 months after the treatment.Although grade was not correlated with presence of androphilic protein,good correlation was observed with androphilic protein detected by R1881 and response to endocrine therapy.

4)Presence of androphilic protein examined by R1881 showed statistically better prognosis.

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STEROID RECEPTORS AND RELATED PROTEINS IN HUMAN PROSTATIC TUMOURS.

P.E.C. Sibley, L. Buttifant, M.E. Harper and K. Griffiths.

Androgen and oestrogen receptor levels in human prostatic tumours have been compared with both histochemical and immunocytochemical determinations of a range of steroid receptors and related proteins. As reported previously, oestrogen receptors were not detected by conventional or immunocytochemical methods but the binding of fluorescent-labelled oestrogens and recently an oestrogen receptor related protein (D5) have been observed in both benign and carcinomatous specimens. Similarly, nuclear androgen receptor levels in both benign and carcinoma samples have not correlated with either the intensity or distribution of fluorescent-labelled androgens, but the clinical relevance of these observations awaits long-term analysis.

In order to identify the moieties, other than specific, high affinity receptors, to which the fluorescent-labelled steroids might bind, human serum albumin has been immunocytochemically localized in 32 prostate tumours. The distribution of this protein was not confined to the blood pool but within the connective tissue compartment of all benign and carcinoma specimens. Indeed, a considerable number of carcinoma cells stained for albumin in both a cytoplasmic and nuclear location but this could not account for the totality of fluorescent-labelled steroid binding.

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ESTROGEN FORMATION IN NORMAL PROSTATE, BENIGN PROSTATIC HYPERPLASIA AND IN EXPRESSED PROSTATIC SECRETIONS.

M.M. Stone, W.R. Fair and J. Fishman.

Estrogen formation in human prostatic tissue was investigated in tissue removed at the time of cystoprostatectomy. Expressed prostatic secretions (EPS) were obtained by digital compression of the prostate. Homogenized tissue and EPS were incubated with either [1,2,6,7-^{3}H]-androstenedione or [1β-^{3}H]-androstenedione in the presence or absence of an aromatase inhibitor, 4-hydroxyandrostenedione (4-OHA). Estrogen formation was determined by reverse isotope dilution of [^{3}H]-estrone and [^{3}H]-estradiol and crystallization to constant specific activity or by the recovery of ^3H_2O. Control incubations were carried out in parallel utilizing heated prostatic tissue.

Total estrogens produced in the periurethral zone in patients with benign prostatic hyperplasia (BPH) was 223 fmoles/mg protein/h (SE ± 57) compared to 102 fmoles in patients without BPH. Estrogen formation in the peripheral zone was 175 fmoles (SE ± 69) and 105 fmoles (SE ± 20) in patients with and without BPH, respectively. Estrogen formation determined by recovery of ^3H_2O was also identified in the EPS at a rate of 10-20 fmoles/h. The prostatic aromatase exhibits Michalis-Menton kinetics with an apparent Km of 90 nM.

4-OHA inhibited aromatization in the prostatic tissue by 57-93%.

These results suggest aromatization of androgens to estrogens in the human prostate and EPS procedes at a substancial rate and that local estrogen formation could preexist and be a factor in the etiology of BPH and prostate cancer The ability of 4-OHA to inhibit prostatic aromatase further suggests a therapeutic benefit may also exist.

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BIPHASIC REGULATION OF PROLACTIN RECEPTORS IN THE RAT VENTRAL PROSTATE

H. Rui, P.A. Torjesen and K. Purvis

Exposure of explants of rat ventral prostates and a rat Leydig cell tumour to ovine prolactin for 20 h caused a dose-dependent biphasic alteration in the subsequent membrane binding of iodinated human prolactin. Low doses (300 ng-3 ug/2 ml) caused an up-regulation of receptor in both tissues whereas higher doses induced a down-regulation. The effect could be obtained with rodent and human prolactin and human growth hormone but not androgens, oestrogens, glucocorticoids or hCG. Up-regulation was initiated between 6-12 h after exposure to hormone, and was independent of prostaglandin synthesis, cAMP and calcium availability. In vivo treatment with high doses of oestradiol valerate for 7 days induced a hyperprolactinemia which was associated with a down-regulation of the receptor.

These results support previous data demonstrating up-regulatory effects of prolactin on its receptor in liver and mammary gland, and the biphasic nature of the response may explain the difficulties reported by other authors to demonstrate alterations in receptor concentration in the prostate after injections of prolactin in vivo.

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THE MEASUREMENT OF ANDROGEN RECEPTORS IN ELECTRO-RESECTED PROSTATIC TISSUE.

I.G. Conn, L.E.F. Moffat, Z. Kirkali, R. Leake, S. Cowan, M. Patel and D. Kirk.

Studies on the suitability of electro-resected prostatic tissue for androgen receptor assay are conflicting (Benson, R.C. et al, Cancer 55: 382-388 1985, Kyprianou, N. et al, Brit. J. Urol. 58: 41-44 1986). Androgen receptors (A.R.) were measured in 25 patients with advanced prostatic carcinoma. In 6, this was by perineal Tru-cut biopsy, during trans-urethral resection in 16 patients, and by both methods in 3. All patients received hormonal treatment following biopsy, and assessment of response to therapy was carried out at 12 weeks. A.R. assay was performed using Mibolerone, a synthetic androgen, as a ligand. Androgen receptor was found in cytosolic or nuclear suspension in 10 of 25 patients. In only 1 of the 3 who had needle biopsy and T.U.R., was there good correlation between the two. Response to therapy was not associated with A.R. status. Both heterogeneity of individual tumours and possible damage to the chips by electro-resection may be the cause of thes conflicting results.

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STEROID RECEPTORS IN HUMAN PROSTATIC CARCINOMA.

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To evaluate the hormone-responsiveness of human prostatic carcinoma (PC),androgen (AR),estrogen (ER) and progesterone (PR) receptors were measured both in the cytosol and nuclear fractions of103 PC.Tissues were collected through transvesical resection and needle biopsy.The exchange assay was performed overnight,at low temperature,using tritiated R1881,17 estradiol,and R5020,in the presence of sodium thiocyanate 0.5 M for ER determination;dextran-coated charcoal method was used.Nuclear fractions were extracted with sodium molybdate 0.2 M.Threshold levels were fixed to 3 fmol/mg protein and 50 fmol/mg DNA respectively for cytosol ER (ERc) and nuclear ER (ERn);to 5 fmol/mg protein and 100 fmol/mg DNA respectively for ARc,PRc and ARn,PRn.

The mean values and the standard errors demonstrated cytosol and nuclear AR and PR content higher than that of ER (ARc=49.26 $\pm$ 11.24 , PRc=76.88 $\pm$ 33.88 , ERc=14.29 $\pm$ 3.39 fmol/mg protein ; ARn=225.61 $\pm$ 25.45 , PRn=220.06 $\pm$ 48.93, ERn=104.87 $\pm$ 16.17 fmol/mg DNA).

The cytosol and nuclear receptors distribution reported in the table,showed a preferential localization of AR and PR in the cytosol fraction.

Table

	cytosol	nuclei
AR^+	78/103 (76%)	26/65 (40%)
PR^+	20/27 (74%)	7/12 (58%)
ER^+	29/51 (57%)	15/33 (45%)

This work was supported in part by a grant of A.I.R.C.

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ANDROGEN RECEPTORS IN TRANSITIONAL CELL CARCINOMA.

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Transitional cell carcinoma (TCC) has been said to be steroid hormone dependent. However, little evidence of response to endocrine therapy is available. We attempted to measure androgen receptor in 16 patients with histologically proven TCC. None of the patients had had previous hormone treatment. Five tumours were Grade I, 3 Grade II and 8 Grade III. Twelve samples were taken by transurethral resection, whilst open resection was performed in the other 4. Receptor was assayed using saturation analysis with mibolerone over the range 0.5-5nM. Excess ORG 2058 eliminated possible binding to progesterone receptor. Significant androgen receptor could not be detected in either the soluble or the nuclear fraction of any of the 16 tumours and it was concluded that, despite possible technical problems, human TCC appears unlikely to be under the direct influence of androgens.

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TOWARDS MONITORING THE "MEMORY" OF IN VIVO 5α-REDUCTASE ACTIVITY IN THE HUMAN PROSTATE - J.M. Le Goff, P.M. Martin, J.M. Brisset, J.M. Husson and J.P. Raynaud

In order to be able to monitor 5α-reductase activity in prostate biopsies from BPH and cancer patients undergoing hormone therapy, we set up a standardized assay in which the chosen conditions respected, as closely as possible, the physiological status of the patient. The samples, immediately frozen in liquid N_2 to preserve the integrity of the ecosystem, were used to prepare a microsomal preparation incubated at 37°C at optimum pH (5-5.5) for a time period that gave rise to less than 10 % of product. Under these conditions the determination of the enzyme concentration (E_o) as given by V_{max} depends little upon the dissociation rate of the substrate-enzyme complex ($V_{max} = k_d E_o$). However, the estimation of the affinity constant K_m by the method of Lineweaver-Burk after incubation in different buffers for short incubation times (3 to 15 mins) gave significantly different K_m values (from 0.48 to 6 uM), for one and the same microsomal preparation, according to the range of substrate (testosterone) concentration used. The rate of dihydrotestosterone formation revealed that the enzyme was unable to adapt itself instantaneously to the in vitro conditions of measurement (hysteresis) but was hyperactive during the shortest incubation times (less than 15 secs). This hyperactivity ("burst") could be explained by a memory of the enzyme for a previous conformation prior to in vitro manipulation and might be the only true reflection of its actual in vivo activity. This phenomenon which argues against the relevance of classical in vitro conditions for the measurement of enzyme activity has already been described for other enzymes.

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5α-REDUCTASE ACTIVITY IN PROSTATE CANCER

A.W.S. Ritchie, F.K. Habib, A. Busuttil, J.E. Newsam & G.D. Chisholm.

The conversion of testosterone to dihydrotestosterone (DHT) is the obligatory step in the mechanism of action of androgen in the human prostate. The enzyme responsible for the production of DHT is the membrane bound 5α-reductase.

This enzyme was measured in TUR tissue from 39 patients with carcinoma of the prostate to test the hypothesis that it is a clinically useful marker of tumour differentiation and response to endocrine therapy.

5α-reductase activity was assayed by thin layer chromatography on 800g supernatants of homogenised tissue after incubation with radiolabelled testosterone and appropriate co-factors. Patients were staged using TNM, followed-up in a designated clinic and assessed using BPG criteria. Histological analysis included Gleason scoring and calculation of the percentage of tissue involved by tumour. Levels of 5α-reductase in the tissue were found to correlate with the sum Gleason scores ($r = -0.45$ $P < 0.01$). This was not due to a positive correlation between 5α-reductase and the percenatge of malignant tissue ($r = -0.48$ $P < 0.01$). Patients with localised disease had significantly higher 5α-reductase levels than those with metastases. Limited follow-up (minimum 18 months) has shown no correlation between tissue enzyme levels and the occurrence or duration of response to endocrine therapy in a selected group of 23 patients.

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THE INHIBITION OF 5αREDUCTASE BY 4-HYDROXYANDROSTENEDIONE.

B. Houston, G.D. Chisholm and F.K. Habib.

4-Hydroxyandrostenedione (4 OH-A) is a potent suicide inhibitor of the enzyme aromatase, and may be useful in the treatment of breast cancer. Recently, Motta (J. steroid Biochem. (1985) 23) has shown that 4 OH-A prevents the formation of DHT from testosterone in extracts of rat and human prostates and has suggested that 4 OH-A may provide a novel approach to the treatment of prostatic disease. We describe here the nature of the inhibition of human prostatic 5αreductase by 4 OH-A. In a series of preliminary experiments the kinetic mechanism of 5αreductase was found to be sequential, with NADPH binding first followed by testosterone. The inhibition of 5αreductase by 4 OH-A was then studied and compared with the parent compound androstenedione (A) and with 4-methoxyandrostenedione (4 MeO-A). All three compounds inhibited the 5αreductase with I_{50} values of: 5×10^{-7}M (A) 1×10^{-6}M (4 OH-A) and 6×10^{-5}M (4 MeO-A). The inhibition by 4 OH-A was competitive with respect to testosterone and non-competitive with respect to NADPH. Similar patterns were obtained for A and 4 MeO-A. These results indicate that these compounds inhibit the rate of formation of DHT simply because they are alternative substrates for 5α reductase. The K_i for 4 OH-A determined in these experiments was 1.5-2.2 uM, which is 1000 times higher than the K_i's of transition state inhibitors of 5αreductase such as 4-methyl-azasteroids. Thus as a 5αreductase inhibitor 4 OH-A appears to have limited potential in the treatment of prostatic disease.

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Regeneration of the thymus in old male rats after orchidectomy: Dose-related suppression of regeneration by testosterone

F.T.A. Fitzpatrick, I.M. Adcock, M.D. Kendall*, M.J. Wheeler** & B.D.Greenstein.

The thymus is a critically important organ during development, but atrophies during adult life, and is generally considered to be unimportant in the immune system of the individual. We have recently found that the thymus, which is virtually undetectable in aging rats (15 months old) is greatly restored by four weeks after orchidectomy. The organ appears to be normal, being lobular, well vascularised and filled with thymocytes. The tissue is well differentiated into a cortex and medulla. The restored thymus may be functioning as part of the immune system once more since the total white cell count was significantly raised (Fitzpatrick *et al*, 1985). We have been able to prevent or reverse the effects of orchidectomy on the thymus with testosterone, and the effect is dose-related. This work may lead to more efficient ways of boosting the immune system, and also points to a possibly important physiological link between the immune and endocrine systems.

Reference: Fitzpatrick, F.T.A. , Kendall, M.D., Wheeler, M.J., Adcock, I.M. & Greenstein, B.D. (1985). J. Endocr. 106, R17-R19.

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HORMONE ACTION AND ONCOGENE EXPRESSION IN THE VENTRAL PROSTATE

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Mechanisms whereby gene expression is regulated in the prostate are being investigated by gene transfer and DNA-hormone receptor binding studies. We have characterised several secretory proteins and the genes which encode them whose expression is stimulated by testosterone. DNA sequences which selectively bind androgen receptor complexes and are essential for gene expression will be described. As with many other specialised genes, androgen-responsive genes expressed in prostatic tissue are regulated aberrantly in cultured cells; therefore we are also employing an alternative strategy involving gene transfer in vitro but growing cells in vivo. Thus we have introduced genes into cells of the urogenital sinus and reimplanted them into animals so that they grow and develop as normal prostate. This approach should be useful for studying the rôle of oncogenes in tumorogenesis as well as hormone action. Preliminary results indicate that the oncogenes v-myc and SV40 large T antigen operate to stimulate the growth of the prostate implant.

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THE EFFECT OF STEROID HORMONES ON THE TRANSFORMING ACTIVITY OF DNA EXTRACTED FROM HUMAN UROLOGICAL TUMOURS

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Previous studies indicated that DNA extracted from human urological tumours could produce only abortive transformation when transfected into primary cultures of mouse embryo fibroblasts (L. Possati et al., Urol Res, in press). In this study we investigated the effect of 17β oestradiol and progesteron on the transforming activity of DNA extracted from human urological tumours, using the same transfection assay as above. Primary cultures of mouse embryo fibroblasts were transfected with DNA extracted from 14 renal cell carcinomas, 1 normal kidney, 12 urothelial tumours and 1 normal urothelium, using the calcium precipitation technique. The cells were also treated with 17β oestradiol or with progesteron simultaneously with DNA and for a week after transfection. Transformation was scored every week evaluating the presence of foci of transformed cells in the transfected cultures and the ability of the transfected cells to form colonies in soft agar. Until now, morphological transformation was seen only in the cultures transfected with DNA from renal cell carcinomas and treated with 17β oestradiol; these cells, however, do not form colonies in semisolid agar medium.

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THE ANDROGEN SENSITIVE HUMAN PROSTATE TUMOR CELL LNCaP: ANDROGEN RECEPTOR FORMS AND THE EFFECTS OF ANDROGENS ON THE RELEASE OF PROTEINS.

E.M.J.J. Berns, W. de Boer and E. Mulder.

Differentation and proliferation rates of human prostate carcinoma are dependent upon androgen stimuli and the hormonal effects are mediated by intracellular receptors. In search for a possible autocrine regulatory function in androgen dependent tumors, we have studied synthesis and release of androgen stimulated protein(s) in androgen sensitive LNCaP cells.
The nuclear extract of the LNCaP cells contained 1,679±558 fmol androgen receptor/mg nuclear extract protein, corresponding to 17,000±2,500 KCl-extractable androgen receptor sites/cell (n=5). Sucrose gradient (high salt) centrifugation revealed two receptor forms sedimenting at 4.55±0.20 and 2.83±0.24 S (n=9), with corresponding Mw of 91 and 33 kD respectively (n=2). Only the 4.5 S form bound to DNA-cellulose. Estrogen and progesterone receptors were not detectable in the nuclear extracts nor in the cytosol. Cells grown in media containing charcoal treated fetal calf serum released significantly lower amounts of several (^{35}S)-methionine labelled proteins, especially of a protein with a Mw of 42 kD. The release of this protein could be restored in cells cultured in the presence of dihydrotestosterone (DHT, 0.1-1μM) or R1881 (0.1nM -0.1μM), whereas estrogens or corticoids and progesterone had no effect.The high concentration of DHT needed to restore this protein is possably related to extensive metabolism of DHT by the LNCaP cells. Anti-androgens, which inhibit cell growth, also exerted inhibitory effects on the release of the 42 kD protein.
The observed correlation between the effects of (anti)-androgens on the growth of the LNCaP cells and the release of the Mw 42 kD protein could be related to the regulation of malignant prostate cell growth.

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ANDROGEN AND GROWTH FACTOR INVOLVEMENT IN PROSTATIC DISEASE.

P. Davies, C.L. Eaton, M.E.A. Phillips and P. Thomas.

Patterns of (proto)oncogene expression have been investigated in benign prostatic hyperplasia (BPH) tissue, human prostate cancer specimens containing or deficient in androgen receptors, and receptor-positive and -negative experimental cell lines. Nuclear androgen receptor concentrations in BPH tissue ranged between 230 and 1350 fmol/mg DNA and those in carcinoma between 0 and 1500 fmol/mg DNA. Transcripts of c-myc, c-H-ras, c-K-ras, c-sis, c-fos, c-myb, c-erb A, c-erb B and p53 gene were found in BPH and carcinoma tissue. p53 was expressed at high levels in all BPH and carcinoma samples. Expression of c-myc was consistently elevated in carcinoma tissue compared to BPH tissue. Expression of c-H-ras and c-K-ras was elevated less consistently. Expression of c-fos was most enhanced in specimens of high androgen receptor concentration, whereas c-sis was most consistently increased in specimens lacking receptor or with very low receptor content. Furthermore, androgen independent cell lines secreted a powerful mitogenic factor of M_r approximately 28000. Most BPH and carcinoma samples contained receptors for EGF on the basis of binding of radioiodinated EGF.

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EVALUATION OF ONCOGENE EXPRESSION IN SIX HUMAN RENAL CELL TUMOR LINES.

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The role of proto-oncogenes in cellular growth, differentiation, and biological development is well documentated. These genes have also been implicated in processes concerning the onset and progression of a tumor. Depending on the predicted function and cellular localization of their gene products, proto-oncogenes can be divided in five different classes. To dermine the potential role of proto-oncogenes of each of these classes in human renal cell carcinoma, we have analyzed 6 human renal cell tumors which were propagated in nude mice, by Northern- and Southern blot analysis, As molecular probes, myc, myb, P53, Ki-ras, N-ras, fes, abl, sis, and fms were used. Using these probes it could be shown that in most of the tumors at least three oncogenes, each representing a subgroup of proto-oncogenes, were expressed at elevated levels. The genetic organization of these proto-oncogenes and there pattern of expression in the renal cell tumor lines will be presented.

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DEVELOPMENT OF ANDROGEN-INDEPENDENT TUMOR CELLS AND THEIR IMPLICATION FOR THE TREATMENT OF PROSTATIC CANCER.

J.T. Isaacs

Nearly all men with metastatic prostatic cancer have an initial positive response to androgen ablation therapy. Essentially all treated patients, however, eventually relapse to an androgen-insensitive state unresponsive to further antiandrogen therapy, no matter how aggressive these additional therapies may be, due to the growth of androgen-independent tumor cells. There are two basic mechanisms for the emergence of androgen-independent cancer cells following androgen ablation. This can occur either by the development during therapy of androgen-independent tumor cells from a pool of initially homogeneously androgen-dependent tumor cells or alternatively by the selective outgrowth (i.e., clonal selection) during therapy of preexisting androgen-independent tumor cells present within an initially heterogeneously androgen-sensitive tumor population. The predominant mechanism for this relapse appears to be the selective outgrowth of clones of androgen-independent cancer cells which preexist within the heterogeneously androgen-responsive prostatic cancers even before androgen ablation therapy is initiated. This preexisting clonal heterogeneity can arise via a series of mechanisms (e.g., multifocal origin, genetic instability). Due to this preexisting clonal heterogeneity, when androgen ablation therapy is used as the sole therapeutic approach, cures are rarely, if ever, achieved no matter how aggressive or complete the androgen ablation. In order to more effectively treat prostatic cancer, additional modalities (e.g., chemotherapy, radiation) specifically targeted at the preexisting androgen-independent cancer cell must be simultaneously combined with androgen ablation. The validity of each of the above points will be demonstrated by a series of animal and human studies.

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CYTOGENETICS OF PROSTATIC CARCINOMA: STUDIES ON TWO HUMAN TUMOR CELL LINES.

Josée J. König, Anne Hagemeyer, Bep E. Smit, Hans C. Romijn and Fritz H. Schroeder.

We studied the karyotypes of two established human prostatic carcinoma cell lines, growing in vivo in nude mice. We made direct preparations of metaphases by injecting colcemid intravenously into the tumor-bearing mouse 1,5 hrs prior to sacrifice. After mincing the tumor and after incubation in collagenase, the obtained cell suspension was further processed for cytogenetic preparation in a standard way. PC-82 is a hormone-dependent and moderately differentiated cell line. It is slow-growing and has a long lag phase after transplantation. The karyotype of PC-82 appeared stable in the different mouse passages studied (27-31). It has a modal chromosome (chr.) number of 85, with 13 consistent markers. The markers were identified as rearrangements of chr. 1, 2, 3, 4, 5, 9, 12, 13, 15 and 17. No normal copy of chr. 1, 2, 12, 13 and 17 was found. PC-133 is a hormone-independent and poorly differentiated cell line. It is relatively fast growing and has a shorter lag phase than PC-82. PC-133 has a modal chr. number of 58 including about 20 markers. The Y-chr. is lost. In further passages, about 5 of the markers showed additional changes, and there was an increase in the number of double minutes. In contrast to PC-82, the karyotype of PC-133 has not yet stabilised. This could be in relation with the faster growth pattern and the more undifferentiated character of PC-133. Identical markers in both cell lines were not seen, although rearrangements of chr. 1, 2, 3, 4, 5, 12, 16 and 17 were found in both tumors.
To learn more about specific chr. aberrations in prostatic carcinoma, not only earlier passages of these cell lines, but also primary patient tumors are at present being investigated.

This project is supported by grant IKR 86-14 of the Dutch Cancer Foundation.(Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands, Dept. of Urology).

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EFFECT OF ESTRAMUSTINE ON TWO SUBCLONES OF LNCAP PROSTATIC CARCINOMA CELL LINE.

M. Hasenson, B. Hartley-Asp, B. Lundh and A. Pousette.

We have investigated the effect of estramustine and steroids on the growth of a hormone sensitive cell line (LNCaP) and a subclone (LNCaP-r) which has been diverged at our laboratory. The effect of the test drugs on cell growth was analysed by cell number and ATP-analysis. The results are as follows:

Substance	Concentration	LNCaP	LNCaP-r
Estradiol	10^{-8}M	Slightly inhibitory	No effect
	10^{-5}M	Strong inhibiting	No effect
5α-DHT	10^{-8}M	Slightly positive	No effect
	10^{-5}M	Strong inhibiting	No effect
Estramustine	10^{-7}M	Slightly inhibitory	No effect
	10^{-6}M	Slightly inhibitory	No effect
	10^{-5}M	Strong inhibiting	Inhibiting
Estramustine + DHT or E_2	10^{-7}M 10^{-5}-10^{-7}M	DHT or E_2 increase inhibiting effect of steroids	No effect
Estramustine + DHT or E_2	10^{-5}M 10^{-5}-10^{-7}M	DHT or E_2 decrease inhibiting effect	

Conclusion: DHT and estradiol affect the growth of LNCaP but not the growth of the LNCaP-r. Estramustine affects both subclones but LNCaP is more sensitive than LNCaP-r. Dihydrotestosterone or estradiol modulate the effect of estramustine on the LNCaP cell line in different ways dependent on the concentration of estramustine.

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BLADDER TUMOR INDUCTION BY CYTOTOXIC AGENTS - EXPERIMENTAL LONG TERM STUDY -

H. Rübben, J. Feichtinger, F.-J. Deutz

Intravesical chemotherapy (IC) is worldwide used to reduce the high frequency of recurrences after complete resection of superficial bladder tumors. Assuming a recurrence rate of 60 %, more than one third of these patients are treated unnecessarily, as they do not develop a recurrence even without chemotherapy. In order to examine the side effects of IC on normal urothelium, 480 female Wistar-rats received adriamycin (ADM) and control substances intravesically, 15 times in a three day interval.

Depending on the concentration of ADM epithelial proliferations and tumors have been induced during a one year follow up in about two percent of the rats.

To check the risk of tumor induction in daily urological practice the experiment was repeated in dogs with mitomycin and cisplatinum. 17 dogs received the drugs intravesically 18 times in a three day interval. All dogs were followed up by cystoscopy, biopsy and cytology. Neither tumors nor carcinoma in situ were observed during a three years observation period.

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TUMOR MARKERS IN UROLOGY

S. von Kleist

It is a fact that there is an urgent need for the improvement of the diagnosis of early cancers, i.e. in a stage when metastases have not yet appeared. Much research effort has been invested for the detection of reliable circulating or cell-bound index substances that could signal if not a primary, at least secondary tumors or metastases before they become decernable by conventional diagnostical means. However, all of the numerous tumor markers that were found, even those more recently characterized by monoclonal antibodies, lack the sensitivity and specificity necessary for a precocious immunological diagnosis of malignancies. Nevertheless, some tumor markers like certain enzymes (e.g. PAP, AP, etc.) and tumor associated proteins (e.g. CEA, PA) are very valuable in common cancers such as carcinomas of the prostate or bladder. However, it is in the already diagnosed cancer patients that they should be employed for the evaluation of the regression or progression of the known malignancies, detection of recurrencies, or detection of therapy resistance and amelioration of the staging of the malignant disease. When detected in the tissue the markers may also help to classify histogenetically the tumor type or identify isolated tumor cells. One of the most important characteristics of tumor markers is that they can add prognostic information to that obtained by the classical staging procedures.

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IMMUNOCYTOCHEMICAL CHARACTERIZATION OF RENAL CELL CARCINOMAS WITH MONOCLONAL ANTIBODIES

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Cryostat sections of 27 renal cell carcinomas (RCC)(21 clear cell, 5 granular cell and 1 sarcomatoid carcinomas) were examined by two-step peroxidase method using epitheliotropic antibodies AK HEA 19, 41, 81, 124, 125, 164, 196; cytokeratin (PKK 1), Vimentin, EMA, TPA; markers of distal (9C2, 25E1) and proximal tubular cells (19G1, 28C1, 35H1, 71C2; Dr. Falkenberg, Bochum) and HLA-ABC and HLA-DR. Results: The epitheliotropic antibodies AK HEA 124 and 125 (24/27) cytokeratin (27/27) and EMA (23/27) as well as HLA-ABC (27/27) and CALLA (24/27) proved to be strong markers. Both HLA-DR and TPA were positive in 11 out of 27 tumours. The co-expression of vimentin and cytokeratin was seen in 22 cases. 26 renal cell carcinomas expressed 1 ouf of 3 antigens of brush-border 19G1, 28C1 and 29H1. Similarly, the combination of CALLA, which shows positivity in proximal tubular cells and 29H1 was positive in 26 specimen. No correlation was observed between morphologic differentiation grade of tumour and antibody positivity. Conclusions: All renal cell carcinomas are cytokeratin-positive, the majority co-expressing vimentin. (Normal tubular cells are positive only for cytokeratin). All renal cell carcinomas are HLA-ABC positive. HLA-DR is expressed by some tumours, predominantly focally. The almost constant evidence of brush-border antigens suggests the proximal tubular epithelium as the origin of renal cell carcinoma and permits its discrimination from other clear cell tumours. The epitheliotropic markers (cytokeratin, HEA's) are helpful in the differential diagnosis of "clear cell sarcomas and carcinomas".

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PROSTATIC SPECIFIC ANTIGENS : A NEW GENERATION OF MARKERS FOR MONITORING PROSTATIC CANCER.

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Serum assays for proteins of prostatic origin, prostatic antigens (PA)(Can.Res.45:886,1985) and γ-seminoprotein (γ-SM)(Jap.J.Clin.Path.32:781,1984) have been developed for monitoring prostatic cancer. We have assessed the Hybritech PSA-RIA, and made a limited comparison with the Chugai γ-SM-EIA. At presentation 27/30 M_1 patients have a PSA>10 ng/ml and 29/30 a γ-SM>10 ng/ml. In M_0 disease 21/61 had a raised PSA and 23/61 a raised γ-SM. PSA and γ-SM levels were found to correlate r=0.68 at presentation. Both proteins are independent of prostatic acid phosphatase (PAP).
Longitudinal studies of 2-4 y showed the independence of PSA and PAP. There was a higher correlation of PSA level and clinical status than with parallel PAP measurements. A PSA >10 ng/ml, with or without a raised PAP, in M_0 patients at presentation carried an increased risk of progression within 2 years. The PSA levels of patients in prolonged remission was generally <5 ng/ml.
A comparison between PSA and γ-SM in 22 patients with M_0 disease showed that both analytes gave a warning of disease progression at a time when PAP levels were normal. PSA and γ-SM were concordant, although γ-SM levels were generally higher.
PSA and γ-SM are both glycoproteins with different molecular weights, 34K and 23K respectively, they show partial immunological identity.

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PROSTATE SPECIFIC ANTIGEN (PSA): EXPERIMENTAL AND CLINICAL DATA.

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Prostate specific antigen (PSA) was investigated for its potential as a prostate tumour marker. A large number of patients with and without prostate disease have undergone serum analysis for Prostatic acid phosphatase (PAP) and PSA with a view of assessing the advantages of PSA as a tumour marker. PSA was purified from seminal fluid and the physico-chemical properties were studied. The protein was found to be more stable in serum than PAP with respect to temperature changes. Purified PSA consisted of isomers with different isoelectric points and these reacted with an antibody directed against the main isomer (MW 36 kD, pI 6.9). In addition PSA binds to chelating sepharose charged with Zn^{2+} ions thus providing a means for its purification by affinity chromatography. Additionally we found PSA to be a more sensitive and specific marker for prostatic cancer than PAP. Furthermore our studies indicated that the measurement of PSA in patients with benign prostatic hyperplasia identified two sub-groups; one group shows PSA values in the normal range (0-5.8 ng/ml) and the other group with higher values ($p<0.001$) ranging between 6.0-12 ng/ml. The latter range coincides partially with values found in patients staged To/T1 (TNM system). We therefore conclude that PSA is a suitable marker for monitoring treatment effects and it is a sensitive probe for the early detection of recurrences in cancer of the prostate.

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SERUM LEVELS OF NEOPTERIN IN PROSTATIC CARCINOMA.

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Neopterin, biosynthesized from guanosine triphosphate in human macrophages, is supposed to represent a stimulation of the immune system. Elevated serum neopterin levels (>10 nM/L) has been found in viral diseases, transplant rejections and in malignant diseases. An activated immune system could be expected to be a part of the defense mechanisms against a tumor, and hence a good prognostic sign. 93 patients with prostatic carcinoma has been studied and serum levels of neopterin were measured before onset of treatment and after 6 and 12 months. (Radioimmunoassay kits from Henning AG, Berlin, FRG). Serum neopterin levels decreased after treatment (orchidectomy or estrogens) within 6 months and persisted lower than initially after 12 months.($p < 0.001$). After 3 years patients with elevated neopterin levels at the time of diagnosis, had a survival rate of 58% compared to 92% still living of those with neopterin <10 nM/L. ($p<0.001$). An evoked immuno defense system as reflected by elevated serum neopterin levels, seems to be a sign of bad prognosis in human prostatic carcinoma, in contrast to what could be expected.

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PINEAL METATONIN- AND PITUITARY HORMONE RHYTHMS IN PATIENTS WITH PROSTATIC CANCER AND IN CONTROLS.

S.H. Fluchter, C. Bartsch, H. Bartsch, A. Attanasio.

A link between pineal gland and cancer has been postulated. Possible mechanisms of action have been discussed.

Serum levels of pineal hormone melatonin (Mel) were measured by RIA at 4-h intervals throughout a 24-h period in BPH-patients (n=13), patients with PC (n=14) as well as in young men (n=10). In parallel measurements of the pituitary hormones prolactin (Prl), growth hormone (GH), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were also carried out by RIA. None of the patients had received previous treatment and they stayed in the same environment. Data were analyzed by the population mean-cosinor method and linear correlation coefficients between the five hormones were calculated for each group.

Mel showed significant circadian rhythms in young men, patients with BPH and incidental PC (PCi, n=5) but not in patients with PC (n=9). 24-h mean concentration (mesor) and amplitude were significantly increased in patients with PC. Prl showed significant circadian rhythms in young men and in patients with BPH, whereas patients with PCi and PC appeared to have ultradian variations. GH did not show significant rhythms in any of the groups. The mesors were elevated in all tumour groups as compared to young men.

The present study suggests that disturbances in the pineal-pituitary-complex may be related to development and growth of PC in man.

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SERUM PROLACTIN (PRL) HAS NO PROGNOSTIC VALUE IN TREATMENT OF CANCER OF THE PROSTATE (CAP).

A. Eriksson*, K. Carlström**, A. Pousette‡ and R. Stege*.

Elevated serum PRL levels in CAP patients treated with orchidectomy or estrogens has been suggested to indicate a poor prognosis (Mee et al 1984). However, estrogen treatment invariably increases PRL levels in CAP patients while orchidectomy has no effect in this respect (Carlström et al 1985). This contradiction prompted us to investigate the relation between therapy response and serum PRL levels in CAP patients in a randomized study.

Eighty patients (54-75 yrs) were randomly allocated to either orchidectomy (N=41) or to estrogen treatment (N=39). Serum PRL levels before and during therapy (months) in responders (R) and non-responders (NR) in the two groups are given in the table (ug/lit, mean and S.E.M.):

		0	6	12	24
Orchidectomy					
R	N=32	5.8 ± 0.5	4.8 ± 0.4	5.8 ± 0.5	6.2 ± 0.6
NR	N= 9	7.7 ± 1.7	6.1 ± 1.0	5.9 ± 1.1	5.8 ± 0.8
Estrogen					
R	N=27	6.0 ± 0.7	12.0 ± 1.5	11.6 ± 1.9	-
NR	N=12	4.6 ± 1.0	14.0 ± 2.1	10.2 ± 1.2	-

We conclude that determinations of serum prolactin have no prognostic value in CAP treatment.

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RENAL CELL TUMOR FLOW CYTOMETRIC ANALYSIS USING CYTOKERATIN AND VIMENTIN AS TISSUE MARKERS.

W.F.J. Feitz, H.F.M. Karthaus, H.L.M. Beck, H. Romein, F.C.S. Ramaekers, G.P. Vooijs and F.M.J. Debruyne.

Nine primary human renal cell tumors (RCT), one lymph node metastasis, four human xenografts of a RCT in nude mice and a rat RCT line were analyzed by flow cytometry (FCM) using propidium iodide for DNA analysis and antibodies to cytokeratin and vimentin in the indirect immunofluorescence technique for labelling of specific tumor cell populations. By means of two-dimensional FCM analysis, vimentin- and cytokeratin positive (tumor) cells could be compared and their DNA content and proliferative fraction could be analysed separately for cytokeratin negative stromal and inflammatory cells. In the primary human RCT two subpopulations of cells can be detected and analysed separately. Co-expression of both cytokeratin- and vimentin type of intermediate filament proteins could be detected in the aneuploid cell population, thus confirming our earlier immunohistochemical findings. Comparison of two model systems of RCT with primary human RCT revealed a similar pattern of tumor cell subfractions within these tumors. In this way additional information of biological properties and potential prognostic values may become available.

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COMPARISON OF THREE DIFFERENT SYSTEMS FOR IMMUNO-HISTOCHEMICAL QUANTITATION OF ABH - ANTIGENS

J. Feichtinger, F. Hofstädter, B. Hufnagel, H. Rübben, G. Jakse

The expression of ABH antigens is correlated with differentiation and prognosis of urothelial carcinoma. However, the simple subjective reading of immunohistochemical staining patterns is difficult because of the very heterogeneous expression of the respective antigens. The borderlines between "positive" and "negative" results are not well defined.

We compared therefore three different methodological approaches for quantitation. The measurements were carried out on sections of paraffin embedded tissue. Immunostaining was performed using monoclonal antibodies against blood group antigens (A,B,H) in an indirect immunoperoxidase technique. Sephadex beads on the respective slides served as an internal standard for the individual staining conditions.

1) Absorption measurements by microdensitometry (VICKERS M 85) at randomly selected areas with the tumor.
2) Morphometric quantitation of weakly, moderate and strong reactions by use of a computerized MOP-System (KONTRON AMO 2).
3) Combined and integrated morphometric and densitometric analysis with an automated TV-image analysis system (Leitz TAS PLUS).

All three approaches are suitable methods for the quantitation of immunohistochemical staining patterns. They could serve as a basis for a more critical definition of staining results.

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URINE-TPA (TISSUE POLYPEPTIDE ANTIGEN) AS A MARKER FOR UROTHELIAL CARCINOMA

B.E. Carbin, P. Eneroth and P. Ekman.

Urine-TPA was measured in 74 patients with bladder cancer. A commercially available RIA-kit was used (Prolifigen® Sangtec, Bromma). Clinical evaluation was also performed including cystoscop, cytology, histology and DNA-measurements. 18 patients had normal cytology, 22 were classified as grade(G) 1, 21 as G2 and 13 as G3 (WHO). The Urine- TPA was elevated in 0, 13, 52 and 54% respectively.
For invasive bladder tumors U-TPA was elevated in 7/15 (47%) pTI-tumors and in 8/10 (80%) if pT≥II.
DNA-measurements showed aneuploidi in 17 patients. 10 had elevated U-TPA (59%).
A well known fact is that U-TPA is elevated in patients with urinary tract infection but we have also recorded elevated U-TPA values in patients treated intravesically with cytostatics.
We conclude that U-TPA is a valuable parameter for monitoring bladder cancer with a 60% sensitivity at a rate of 4 % false positives.

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A NEW MARKER FOR TESTICULAR CANCER.

Su Metcalfe and Karol Sikora.

We have studied patients with testicular tumour and compared the standard markers AFP and BHCG with a new haemagglutination test for tumour presence. B5 is a rat monoclonal antibody which has been found to haemagglutinate erythrocytes from patients with malignant disease. The incidence of B5 positivity is about 80% in cancer patients which, when compared to a normal incidence of about 20% in control groups, implies that people who are normally B5 negative become B5 positive if they develop cancer. B5 haemagglutination was tested in patients attending the testicular tumour clinic over a period of two years. We found that a) both teratoma and seminoma patients were B5 positive: b) individual patients revert to a B5 negative state when the tumour is successfully removed: and c) some patients remain B5 positive although clinically without active disease; this group is considered to represent those individuals who are naturally B5 positive, although the possibility of persistent disease should be considered (as was subsequ ently found with two patients in this study). Comparison of the B5 results with those for AFP and BHCG showed that the incidence of marker positivity at diagnosis was: for teratoma: AFP 12/18; BHCG 14/18; and B5 17/18; for seminoma: AFP 0/10 ; BHCG 4/10 ; and B5 10/10. Monitoring of 79 individuals has involved 425 B5 tests to date, and B5 has been more sensitive to tumour presence than AFP or BHCG for some individuals with teratoma, with a small tumour load at presentation; and others with recurrent disease. For seminoma, the B5 test was of particular value in the absence of other proven markers.

We conclude that there is a role for the B5 test to be included in routine monitoring of testicular tumour patients.

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AN EVALUATION OF A PROSTATIC SPECIFIC ANTIGEN ASSAY, USING A COMMERCIALLY AVAILABLE KIT, IN PROSTATIC DISEASE.

P.G. Ryan, H. Hughes, M. Penney, and W.B. Peeling

Commercially available assays for serum prostatic specific antigen (PSA) have only recently become available. We have evaluated the Hybritech double monoclonal antibody assay for PSA in prostatic disease.
Initial experiments were performed to establish the stability of PSA in blood for this assay, and to confirm the in-batch and between-batch precision of the assay. Results confirm PSA to be stable for up to 24hr at room temperature, and for results to be reproducible both in-batch and between-batch, with a coefficient of variation <4.5% for PSA levels above the normal range of 0.1-2.7 µG/L.
Several groups of patients with prostatic cancer have been followed longitudinally with serum PSA assays. The results suggest a high correlation between PSA levels, trends in serial PSA's, and the clinical status of the patient. This occured both with 1st and 2nd line treatment regimens. A rising PSA also provided early objective evidence of disease progression. These results suggest that PSA measured by this technique is reproducible, unaffected by delay in performing the assay; and is useful in the diagnosis and follow up of patients with cancer, possibly providing an early objective indication of disease progression and thus enabling early management changes to be initiated to the patients benefit.

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BPH CELLS IN PRIMARY CULTURES: A POSSIBLE MODEL FOR DRUG RESPONSE IN HUMAN PROSTATIC CANCER [CAP]

N. Deshpande, R.C. Hallowes and J.M. Towler

In CAP, there are no established laboratory tests which will predict the likely response of a drug prior to its administration. Since pure malignant tissue cannot be obtained from TUR specimens, we decided to use BPH tissues to evaluate the effects of various hormonal treatments used to treat patients with CAP and interferons, on the activities of certain enzymes of carbohydrate metabolism. Thus BPH cells were obtained after collagenase digestion and were grown on collagen gel. The primary cultures were then treated with androgens, oestrogens, medroxy - progesterone acetate, LH-RH and interferons for 72 hours and the activities of eight enzymes of carbohydrate metabolism were estimated biochemically.

The results show that testosterone propionate inhibits the activity of α-glycerolphosphate dehydrogenase and hormonal drugs and interferons are capable of overcoming the androgen induced inhibition. Since the ability of some of these drugs to produce responses in CAP is well established, our findings indicate that the likely response might be predicted by such an in vitro test. Furthermore the test can only predict the direct action of a drug on the prostatic tissue since LH-RH which acts indirectly was without any effect in our model. It will be interesting to evaluate these findings clinically.

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SERUM LEVELS OF THE "STEROID SENSITIVE" LIVER PROTEIN PREGNANCY ASSOCIATED α_2-MACROGLOBULIN (α_2-PAG) AND GROWTH HORMONE (GH) IN PROSTATIC CANCER (CAP) PATIENTS DURING PARENTERAL AND ORAL ESTROGEN TREATMENT.

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Several side effects of oral estrogen therapy in CAP are related to liver infection. However, sole parenteral estrogen administration to postmenopausal women is reported to be without biochemical liver effects (Holst et al 1983). We have studied the effects of sole parenteral and parenteral + oral estrogen therapy in 26 CAP patients (60-84 yrs) upon the serum levels of α_2-PAG, which is the most sensitive marker of estrogen effects upon the liver, and of GH, a hormone known to profoundly influence liver function. 13 patients (group 1) were given 160 mg polyestradiol phosphate (PEP) i.m. monthly and 150 µg ethinylestradiol (EE) per os daily during a treatment period of 6 months. Another 13 patients (group 2) were treated similarly, but oral EE was given only during the last three months.

In group 1 α_2-PAG and GH levels were highly significantly elevated after 3 and 6 months of treatment. In group 2 neither α_2-PAG nor GH were altered by the sole parenteral treatment (3 months), but were significantly elevated when oral EE was added (6 months).

I.m. PEP treatment in CAP results in very high peripheral estradiol lavels (14.000 pM; Leinonen et al 1979). Despite that, α_2-PAG and GH levels remained unaffected. This further indicates that parenteral treatment does not involve hepatic side effects. The different responses in GH upon parenteral and oral estrogen indicate that the effect of oral estrogens upon the liver may be mediated via increased GH-levels.

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THE USE OF LECTINS TO DEMONSTRATE MORPHOLOGICALLY IDENTICAL BENIGN PROSTATIC CELLS AND CHANGES IN CARBOHYDRATE EXPRESSION IN MALIGNANCY. P. Abel, A. Leathem, A. Ayott and G. Williams.

Carbohydrates (CHO) are expressed on cell surfaces and alter during normal development and in malignancy. CHO expression is a reflection of enzyme activity controlled by each individual cell. Morphologically identical cells may, therefore, have different cell surface CHO's.

Forty specimens of non-malignant prostate and sixteen carcinomas were studied following formalin fixation and paraffin-embedding, using lectins to demonstrate CHO binding sites. Lipid solvents were used during processing and, therefore, only glycoprotein based CHO structures were assessed. For benign prostate, eight lectins were used. One lectin has been used on the carcinomas to date.

In all benign specimens studied, Concanavalin A (Con A), Wheat-germ (WGA) and peanut (PNA) bound over 90% of epithelial cells. Ulex, Gryphonia, Soya bean, Dolichos and Bauhinia bound up to 10% of epithelial cells including some stained by Con A, WGA and PNA but also identifying other sub-groups of cells. Some cells showed no binding to any of the lectins used. Con A and WGA also showed major stromal binding. The other six lectins showed no stromal binding. Hyperplastic cells stained more intensely than normal cells. Patterns of expression were otherwise similar in all cases.

In prostate cancer, Ulex bound to over 95% of malignant cells. Previous studies using frozen sections of benign prostatic tissues indicate that the Ulex receptor is predominately lipid-based. This present work suggests that the Ulex receptor becomes protein based in malignancy and this may be useful as a tumour marker.

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CHARACTERISATION OF AN EPITHELIAL GROWTH PROMOTER DERIVED FROM A HUMAN PROSTATIC CARCINOMA CELL LINE (PC3). C.L. Eaton, P. Davies, M.E.A. Phillips & P. Thomas.

The involvement of protein and steroid hormones in the growth regulation of a high proportion of prostatic tumours is well documented. Control of tumour growth by other factors, in particular those associated with prostatic tumours themselves - autoregulatory tumour products or inductive products of adjacent cells - are less well defined. This is due in part to the limited availability of prostatic tumour cell culture systems and also to the lack of suitable normal epithelial cell lines to facilitate the characterisation of both neoplastic and non-neoplastic secretory products. Recent studies (E. Simpson et al Endocrinol. 119 1615 1985) have suggested that the human prostatic carcinoma cell line PC3, secretes an osteoblast-stimulating factor. The promotion of prostatic tumour growth in bone has been suggested as a possible role for this secretory product.

In the present study we have examined medium conditioned by PC3 cells for its capacity to alter epithelial proliferation rates in cell culture using a normal canine prostatic epithelial cell line (CAPE 1) in a bioassay system. PC3 conditioned medium displayed powerful, concentration dependent growth promoting activity in rapidly growing (log phase) CAPE cell populations. This mitogenic activity has been further characterized by fractionation of conditioned medium. Preliminary studies suggest that secretory and growth promoting activities are associated with fractions of moderate molecular weight (>25K). The presence of such epithelial growth promoters associated with an androgen independent cell line (PC3) suggest an obvious autoregulatory function in this tumour, while the profound effects in normal prostatic epithelial populations may have aetiological implications for prostatic cancer.

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CORRELATION OF ENDOGENOUS TISSUE HORMONES AND QUANTITATIVE MORPHOLOGICAL (STEREOLOGICAL) FINDINGS IN NORMAL AND HYPERPLASTIC HUMAN PROSTATES.

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In previous light and electron-microscopic analyses human benign prostatic hyperplasia was shown to be predominantly a stromal disease; the aim of the present study was to correlate the stereological data and the levels of the endogenous tissue hormones (androgens, estrogens, progesterone) of normal (N) and hyperplastic human prostatic tissue (BPH). BPH tissue specimens were obtained by open prostatectomy (n=25); normal prostatic tissue was obtained from kidney donors (n=5).

No statistically significant difference was found between normal and hyperplastic tissue. Testosterone: BPH=0.69 ± 0.44; N=0.23 ± 0.13; 5 -dihydrotestosterone: BPH=6.1 ± 1.7; N=4.2 ± 0.7; progesterone: BPH=0.059 ± 0.022; N=0.061 ± 0.005; estrone: BPH=0.10 ± 0.03; N=0.14 ± 0.04; estradiol: BPH=0.006 ± 0.02; N=0.04 ± 0.02; estriol: BPH=0.02 ± 0.01; N=0.04 ± 0.03.

Using the Spearman rank correlation coefficient a statistical analysis was performed for age, weight of the prostate, absolute stereological data and the endogenous prostatic hormones. As can be seen from the statistical analysis there is a poor correlation for 5 -dihydrotestosterone and the amount of the glandular epithelium; otherwise no correlation of the endogenous tissue hormones with the stereological data investigated was found.

These data show that the stromal overgrowth of benign hyperplasia is not reflected in the tissue hormone levels.

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PROSTATIC EPITHELIUM INHIBITING FACTOR: ORGAN SPECIFICITY AND PRODUCTION BY PROSTATIC FIBROBLASTS.

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Previous work showed that prostatic fibroblasts (PF) secrete a factor into the culture medium which inhibits clonal growth in soft agar of several prostatic epithelial cell lines. The inhibitory effect is most prominent at the level of DNA synthesis. Consequently, in the present study conditioned media (CM) of PF were tested for their capacity to inhibit DNA synthesis using a micro ^{3}HdT incorporation assay. The results so far obtained are in agreement with previous findings, i.e. CM of BPH and prostatic carcinoma fibroblasts inhibited DNA synthesis, whereas CM of normal PF had no or marginal activity. Further characterization showed: 1) cell lines of non-prostatic origin (K562, MCF7) did not react to CM of PF at all, demonstrating the specificity of the factor for prostatic epithelial lines; 2) CM of skin fibroblasts tended to exert a stimulatory effect, suggesting that the factor is exclusively secreted by PF; 3) the inhibitory effect was not reversible and tended to increase when cells are exposed longer to CM; 4) treatment of CM for 1 hr at 60°C. did not impair activity, indicating that the factor is quite stable; 5) fibroblasts grown on medium depleted of steroid hormones continued to shed the factor in nearly the same amount, suggesting that the in vitro synthesis is androgen independent; 6) inhibiting activity could be precipitated by 60-80% ammonium sulphate.
A homogenate of BPH tissue exerted a very large inhibition. It remains to be investigated whether this inhibitory effect can be ascribed to the same factor as secreted by PF in monolayer culture.

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PROLIFERATION AND SECRETORY FUNCTION OF RAT VENTRAL PROSTATE IN ORGAN CULTURE

P. Martikainen, S. Mäkelä, P. Härkönen and J. Suominen

Organ culture of rat ventral prostate in defined medium was used as a model to study the multihormonal control of the prostate. By a morphometric method the necessity of T (testosterone 10^{-7}M), I (insulin 0.08 IU/ml) and C (corticosterone 10^{-7}M) for the morphologic integrity was shown. The role of these hormones on cell proliferation was further studied by quantitating ^{3}H-thymidine incorporation and by ARG, and on the secretory function by measuring prostatein, the major secretory protein, in culture medium. The de novo synthesis of proteins was studied by fluorography of (^{35}S)-methionine labelled proteins.
T was shown to increase thymidine incorporation of cultured prostate by about 50%, I alone by 100%, and when combined with T, maximal stimulation by more than 300% was achieved. In ARG the labelling of epithelium was increased by T and I, locating mainly on basal cells, but I in addition extended the label over the entire epithelium when observed for one week, indicating thus a shortened cell cycle. C, on the other hand, had a strong inhibitory effect in all culture experiments. Prostatein accumulated during the first week in culture medium in considerable amounts even without hormones, which was propably due to emptying of epithelial cells and acini from the pre-existing secrete. During the second week, T was a prerequisite for prostatein accumulation in medium, indicating that prostatein synthesis was androgen-dependent, which could also be seen from fluorography of the de novo synthesized proteins. I plus T during the second week significantly decreased the amounts of prostatein secreted in culture medium, whereas C plus T increased the secretion throughout the culture. It may be proposed that the high proliferation rate of epithelial cells may prevent or retard the differentiation of the cells.

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ZINC, CADMIUM AND SELENIUM CONCENTRATIONS IN SEPARATED EPITHELIUM AND STROMA FROM PROSTATIC TISSUES OF DIFFERENT HISTOLOGY.

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Our previous studies have shown a distinct biological antagonistic effect between Zn and Cd in the prostatic gland. Selenium has the possibility to inhibit carcinogenisis in several animal systems (D. Medina, C.J. Oborn, Cancer Res. 41: 4386, 1981). The stromal-epithelial interaction in the prostate is important in the formation and accumulation of DHT. We therefore decided to measure the concentrations of Zn, Cd and Se in separated stroma and epithelium obtained from normal BPH and cancerous prostate tissues with a view of assessing the impact of tissue histology on the trace element concentrations; these measurements were compared to those obtained from whole tissues. The separation of stroma and epithelium was done with collagenase (SERVA). The different fractions were wet ashed with nitric acid and analyzed by flameless A.A.S. The zinc concentration in both the stromal and epithelial fractions of BPH is increased compared with those of normal prostate. The zinc values of carcinoma tissues are decreased in both fractions. Cadmium is increased in the epithelium of normal prostate and BPH we found. In the whole prostatic carcinomatous tissues the Cd levels are distinct higher than in the other ones. It seems that the Cd-binding is favoured in the stromal fraction of carcinoma. The Se concentration in the stroma do not show distinct differences in all tissues. The epithelial levels on the other hand are increased in carcinoma. It seems that an interaction of selenium and cadmium in the prostatic epithelium of carcinoma exist.

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EFFECTS OF SEX HORMONES ON EPITHELIAL CELLS ARE MEDIATED VIA TROPHIC INFLUENCES FROM STROMA
G.R. Cunha

Many responses to sex hormones in epithelia of the male and female genital tracts appear to be mediated via connective tissue of reproductive organs. This concept is supported by several observations: In neonatal mice estrogen stimulates uterine epithelial growth and causes epithelial hypertrophy. However, in neonatal mice 1 to 5 days old these estrogen responsive uterine epithelial cells lack nuclear estrogen binding sites as judged autoradiographically and whole cell uptake of radiolabelled estradiol verifies the absence of specific, high affinity, saturable estrogen binding in these uterine epithelial cells. In contrast, the neonatal uterine mesenchymal cells possess true estrogen receptor activity. In developing mouse fetuses, prostatic epithelial development is induced by urogenital sinus mesenchyme (UGM) in response to androgens. Autoradiographic localization of ^{3}H-DHT demonstrates androgen receptors within mesenchyme cells and their apparent absence in the epithelium. Moreover, since prostatic development is induced in tissue recombinants composed of wild-type UGM + Tfm (testicular feminization) bladder epithelium (BLE) it is evident that an androgen-receptor-deficient, androgen insensitive Tfm epithelium can exhibit androgen-induced prostatic ductal morphogenesis, growth, and secretory cytodifferentiation. All of these androgen-induced effects are expressed within Tfm prostatic epithelium lacking androgen receptors. Thus, the presence of the intra-epithelial androgen receptor is neither necessary or sufficient for induction of prostatic epithelial DNA synthesis by androgen. These observations coupled with the observations that neither estrogens nor androgens have direct mitogenic effects on primary cultures of their target epithelial cells provides considerable evidence supporting an indirect, stroma-mediated mechanism of hormone action upon epithelial cells.

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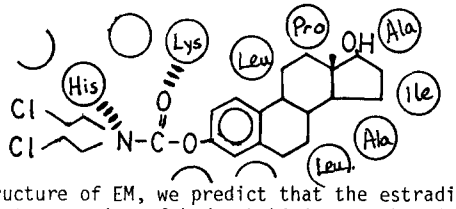
THE MECHANISM OF ACTION OF ESTRAMUSTINE. Kenneth D. Tew and Mark E. Stearns

Estramustine (EM) combines estradiol and nor-nitrogen mustard via a 'peptide-like' carbamate-ester linkage. The drug was designed to enhance cellular uptake via steroid receptors with a subsequent intracellular release of the alkylating mustard. However, EM exhibits neither hormone nor alkylating activities, but instead has a cytotoxic mechanism related to its binding to microtubule associated proteins (MAP's). MAP's are an integral microtubule component, important for microtubule stability and related intracellular transport processes. Using either ^{3}H-EM or a fluorescent analogue of EM (dansylated-EM), we have demonstrated non-covalent binding of the drug to a MAP of 270 KD in human prostatic carcinoma cells, 300 KD in squirrel fish erythrophores and to dynein (a MAP with ATPase activity) in sperm axonemes. Drug binding to these proteins caused cytoskeletal breakdown and cessation of axonemal beating (sperm).



From the structure of EM, we predict that the estradiol moiety binds to a region of hydrophobicity on the MAP-molecule, whilst the carboxyl and tertiary nitrogen allow hydrogen bonding to contiguous amino acid residues. Such interactions could be compared to the binding of a simple dipeptide and would prevent the MAP from recognizing its normal microtubule binding sites.

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TREATMENT OF ADVANCED PROSTATE CANCER WITH DECAPEPTYL^{R} - A NEW SLOW-RELEASING DEPOT OF A LHRH-ANALOGUE
K. Kleinschmidt, I. Papadopoulos, L. Weißbach

In a prospective clinical study 30 patients with histologically verified advanced prostate carcinoma were treated with the LHRH-analogue Decapeptyl^{R}. The micro-encapsulated depot was given intramuscularly in intervals of 4 weeks.

24 patients had measurable tumor-related parameters. 6 patients who refused to undergo castration had locally advanced tumors. Measurable metastaseses and cytological regression by aspiration biopsy were evaluated after 3 and 6 months. The hormone profiles of Plasmatestosterone, FSH and LH were recorded during treatment. The median observation period was 6 months.

According to the criteria of the NPCP, partial remission occured in 16 of the 24 patients with measurable tumor-related parameters and 2 patients had a complete remission. In 2 cases stable disease could be achieved; in 4 cases progression was observed. The Plasmatestosterone level was suppressed to castration standard in all patients within 4 weeks. The following side effects were noted: hot flushes as well as loss of libido and of erectile function. The local tolerance of the LHRH-depot was very good. The patients' compliance was 100 %.

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DECAPEPTYL (D-TRP-LH-RH SLOW RELEASE) IN PATIENTS WITH PROSTATE CARCINOMA STAGE C AND D.
H. Knönagel and D. Hauri.

Since 1984 25 patients with newly detected, advanced prostate carcinoma have been treated with Decapeptyl. An injection of 3 mg i.m. was given monthly. The treatment lasted three months at least. The basal plasma testosterone decreased in all cases to the level of castration within two or three weeks and has not increased again after a period of 18 months.

The prostate carcinoma showed a partial regression in 50% of the patients, was stable in 35% and a progression happened in 15%.

Three patients had stopped the treatment. Their plasma testosterone returned to pretreatment levels within six weeks and consequently sexual function recovered too.

The patients with progression had undifferentiated carcinomas and their prostate acid phosphatase was not raised either.

The treatment with Decapeptyl seems to have the same effect on the prostate carcinoma as the withdrawal of hormones by orchiectomy. Its great advantage is reversibility: Patients, who do not respond to a probationer therapy, have not to undergo castration.

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CAN PRETREATMENT SERUM TESTOSTERONE PREDICT RESPONSE TO ANDROGEN DEPRIVATION THERAPY? D.P. Hickey and M.S. Soloway

Since 1941 androgen deprivation has been the standard treatment for metastatic prostate cancer. However, up to 20% of patients fail to respond to this form of therapy and have a poor prognosis. Identification of the non-responding group prior to commencement of androgen deprivation would allow earlier institution of alternative therapy.

Sixty-five patients with stage D2 carcinoma of the prostate confirmed by tissue biopsy and a positive bone scan were initiated on various forms of androgen deprivation therapy. Twenty-three received Buserelin, 200 mcg/day, 19 DES 1 mg t.i.d., 6 Megace 160 mg q.i.d., 2 Leuprolide 1 mg/day, 9 had an orchiectomy, 3 Zoladex 3.6 mg q 28 days and 3 Naferelin 300 mg b.i.d. There were 3 (5%) complete responses, 18 (27%) partial responses, 31 (4%) remained stable, and 13 (20%) progressed. Pretreatment serum testosterone ranged from 150 ng/dl to 879 ng/dl. The mean serum testosterone in patients who subsequently had a complete response was 524 ± 18.04 ng/dl. The mean level in those who progressed was 285.0 ±110.1. These two groups could not be compared statistically because of the large standard deviation in the progression group. However, of the 16 patients who had a pretreatment serum testosterone of greater than 500 ng/dl only 1 (6%) progressed. Conversely no patient with a pretreatment serum testosterone less than 200 ng/dl had a complete or partial response.

This analysis suggests that pretreatment serum testosterone may predict the probability of a poor response to ADT and identify patients whose primary treatment should be chemohormonal.

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CONSIDERATIONS IN THE USE OF ANTI-ANDROGENS.
H.J. de Voogt, B.R. Rao, L.G. Gooren and F.G. Bouman.

Since Huggins' demonstration that androgen deprivation causes prostate atrophy and reduction in tumorburden in prostate cancer patients a number of variations in therapy have been tested. In recent years attention has shifted from orchidectomy and oestrogens to the use of antiandrogens, which block androgen action mediated via androgen receptors. Their use in combination with LHRH-agonists is a more recent development.

We evaluated the effect of Anandron (RU 23908) on endocrine parameters in normal, healthy males. Serum-T-, LH- and FSH-levels and Prolactine were measured before and after 8 weeks of Anandron treatment. In some of them transrectal ultrasonography of the prostate was done before and after this full course of antiandrogens and after prolonged estrogen treatment. A significant increase in T- and LH-levels was observed, while FSH and Prolactine only showed minimal variations following Anandron treatment. In spite of this increase clinically all subjects showed signs of feminisation, such as gynaecomastia, loss of hair growth and loss of libido. Remarkably there was no difference in size and volume of the prostate, following Anandron treatment, as well as after prolonged estrogen treatment. Histology of prostate biopsies however showed a complete atrophy or even absence of glandular tissue and the presence of increasing amounts of stroma.

These observations support the hypothesis that Anandron is a potent antiandrogen in humans and increase in T- and LH-levels are indictative of this action.

If T-levels should decrease during the course of antiandrogenic treatment this might be suggestive for a failure of anti-androgenic action.

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COMPLETE ANDROGEN BLOCKADE : CLINICAL CONFIRMATION OR REFUTATION OF A HYPOTHESIS - J.P. Raynaud

The concept of complete androgen blockade (inhibition of the effects of both testicular and adrenal androgens on the prostate) continues to be the subject of hot debate despite confirmation of the hypothesis in an animal model and despite the encouraging results published for open and controlled clinical trials. The antiandrogen Anandron blocks the effect of exogenously administered adrenal androgens on the prostate in the rat (a species with very low adrenal androgen secretion). In 3 multicentre double-blind clinical trials statistically significant improvements have been recorded in previously untreated patients with stage D_2 prostate cancer who received Anandron directly after castration for 6 months or more. The extent to which available pharmacological, kinetic and clinical data on Anandron support or refute the hypothesis of complete androgen blockade will be considered. The discussion will be centered on the interpretation of the results of receptor and enzyme assays on prostate tissue and plasma assays of active drug, LH, FSH, prolactin, testosterone, estradiol, cortisol, adrenal androgens (DHEA, DHEA-S, Δ 4-androstenedione).

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COMPARISON OF VARIOUS ANDROGEN BLOCKING REGIMENS ON TISSUE DIHYDROTESTOSTERONE(DHT) IN BPH PROSTATES
J. Geller, J.D. Albert and W. Fay

DHT is the major stimulus to growth of prostate epithelial cells. We have compared a variety of regimens in regard to ability to lower prostate DHT since such a comparison would provide a basis for the best rational therapy in metastatic prostate cancer. Patients with BPH were treated for one week prior to surgery with either Tamoxifen (TM), flutamide (F), megestrol acetate (MA), MA plus TM and MA plus diethylstilbestrol (DES) or MA plus ketoconazole (KC) and mean prostate DHT for each group compared with DHT values for a control (C) group who received no therapy. Whole tissue obtained at the time of surgery was assayed for DHT; plasma T was also assayed. The effects of these various drug regimens in 81 patients are summarized in the Table.

RX	NG DHT/G±SE	N	P(vs.C)	P(vs.MA)	P(vs.MA+DES)
C	6.00±0.45	25	--	<0.001	<0.001
TM	5.83±0.60	7	>0.8	<0.001	<0.001
F	3.89±0.58	12	<0.02	<0.001	<0.001
MA+TM	1.77±0.29	6	<0.001	>0.3	>0.05
MA	1.37±0.06	23	<0.001	--	>0.1
MA+DES	0.88±0.29	4	<0.001	>0.1	--
MA+KC	0.84±0.12	4	<0.001	<0.05	>0.9

It appears that MA plus DES and MA plus KC decrease tissue DHT more than the other treatments. The MA plus DES therapy is being evaluated clinically, since the effectiveness of antiandrogen therapy for androgen-dependent prostate cancer may be inversely related to prostate levels of DHT.

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ADRENAL BLOCKADE WITH AMINOGLUTETHIMIDE IN PROSTATE CANCER.

R. Murray and P. Pitt

The optimum treatment for patients with advanced prostatic cancer who have relapsed following a remission to, or failed to benefit from orchidectomy or oestrogen treatment is uncertain. We present here results of treatment with aminoglutethimide (A/G) and physiological steroid replacement in 127 men (median age 69 years range 50-89) with advanced prostatic cancer which was resistant to orchidectomy and/or oestrogen therapy. Classification of response was according to NPCP criteria. All patients had had a prior orchidectomy and/or oestrogen therapy and all patients had actively progressing symptomatic disease. Most had also had prior radiotherapy. Twenty (16%) patients had an objective remission while 27 (21%) had stabilization of previously progressing disease. Performance status (ECOG) significantly improved in these groups while it significantly decreased in the group with progressive disease. Mean survival was significantly longer ($p < 0.001$) in the remitters (16.2 months) and the static group (8.5 months) than in the patients who failed to benefit (4.7 months). Side effects were minimal and the drug was ceased because of toxicity in only one patient.

Conclusions

1. Adrenal blockade with A/G and steroid replacement is a safe and useful treatment in patients with advanced prostatic cancer who have failed standard therapy.
2. Approximately 37% of patients have an improvement in performance status and survival.
3. Earlier treatment with A/G might lead to better results and warrants investigation.

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ULTRASTRUCTURAL DETERMINATION OF ELEMENTS IN TRANSITIONAL CELL CARCINOMA (TCC) OF THE URINARY BLADDER

R. Friedrichs, W.-G. Burchard, H. Rübben

Elements can be determined in cells by ultrastructural methods combined with measurement of x-ray emission. The objective of the present study was to compare the concentration of elements in normal urothelium and TCC of different stage and grade.

Specimens of normal urothelium and TCC obtained at TUR from 22 patients were immediately fixed in glutaraldehyde at 4 °C for 2 h. After fixation the tissue was dehydrated in alcohol and embedded. Sections (15 u) were cut, mounted on carbon grids, and then coated in vacuo with carbon. For measurement of x-ray emission (x-ray microanalysis) the sections were processed through a scanning and transmission electron microscope with an energy dispersive type x-ray microanalyzer. The determination of elements was performed by point analysis.

The preliminary data suggest that the concentration of elements in TCC differs from normal urothelium. Also differences between tumors of different stage and grade are found. The results may bee explained by the replacement of the asymmetric unit membrane by a non-specific membrane in TCC. This membrane lacks the specialized properties of impermeability, and the cells are exposed to the high concentration of ions in urine.

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ALLERGIC MANIFESTATIONS OF MITOMYCIN C

J.A. Inglis, D.A. Tolley, K.M. Grigor

Eosinophilic cystitis is a rare form of vesical inflammation first reported in 1960 and seldom recorded since (E.W. Brown, J. Urol. 83, 665, 1960).
In the past, food allergy, systemic infections and parasitic agents have all been attributed causes.
Bladder biopsy in affected cases characteristically shows infiltration through all layers with eosinophils (R.H. Littleton, J. Urol. 127, 132, 1982).
We report three patients with proven superficial bladder cancer who have been treated with bladder instillations of the alkylating agent Mitomycin C and in whom successive instillations have been followed by progressively more severe symptoms of vesical irritation with subsequent development of an urticarial rash typically affecting the palms and soles of feet as well as face and back in one case.
Urothelial histology obtained acutely, confirmed eosinophilic infiltration while both symptoms and pathological abnormalities disappeared on withdrawal of the drug. Symptoms of vesical irritation and palmar rash have been previously described and attributed to chemical cystitis and/or topical contamination after voiding (I. Nissenkorn, J. Urol. 126, 596, 1981) but our findings suggest an acquired allergy to Mitomycin C as the underlying cause. The three cases' presentation and management are submitted. We conclude from their reaction to treatment and subsequent progress that symptoms of cystitis and/or rash following Mitomycin C justify cystoscopy and biopsy before further instillation is contemplated. Severe reactions to vesical instillation may alter host/tumour response and affect prognosis.

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FREQUENCY AND DYSPLASIA OF BLADDER EPITHELIUM

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Among 150 patients with negative urine cytology (routine and bladder washing) examined for unexplained frequency a group of 10 patients (7 men, 3 women) has been identified. Their bladders were explored according to a standard protocol which includes : urine cytoflowmetry, chromocystoscopy, cystoscopy under general anesthesia with bladder overdistension and bladder biopsy.

The primary criteria of epithelial dysplasia are lack of cytoplasmic clearing, crowding of nuclei, lack of normal polarisation of cells. The secondary criteria are increase in the size of nuclei, modification of the chromatin pattern, presence of large nucleoli. Of less interest is the presence of mitoses and notching of nuclei.

Among the patients with severe and mild urothelial dysplasia 5 had positive cytoflowmetry (more than 10% of hyperdiploid cells), 5 had negative. The patients with positive cytoflowmetry had been considered as having bladder carcinoma in situ and had been treated by local BCG or mitomycine therapy. For the others niether the diagnosis nor the treatement is clear as yet.

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PERIOPERATIVE IMMUNE PROFILES IN PATIENTS WITH RENAL CELL CARCINOMA

V. Daniel, S. Pomer, G. Opelz, K. Dreikorn, L.Röhl

T-lymphocyte subsets and skin test reactivity was studied in 85 patients with renal cell carcinoma. T-lymphocyte subsets were determined by flowcytometry with the monoclonal antibodies OKT4 and OKT8. Skin reactivity was measured by multitest Merieux. Mean value of T4/T8 ratios before tumor resection was 1,7 ± 0,8 (controls: 1,7 ± 0,6). 17 % of tumor patients had decreased ratios <1,0 in contrast to 3 % in normal controls ($p < 0.0005$). T4/T8 ratios after tumor resection increased from 1,6 ± 0,6 (N=32) to 1,8 ± 0,7 (N=15) and 2,0 ± 0,6 (N=10) when controlled three times postoperatively. T-lymphocyte subsets were not correlated with tumor spread (T1: 1,5 ± 0,4 (N=3); T2: 1,6 ± 0,6 (N=30); T3: 2,0 ± 1,2 (N=27); T4: 1,5 ± 0,6 (N=18), lymph node involvement (N0: 1,7 ± 0,9 (N=60); N1: 1,5 ± 0,5 (N=10); N2: 2,1 ± 1,3 (N=6) or metastases (M0: 1,7 ± 0,8 (N=53); M1: 1,7 ± 1,0 (N=21); M2 1,3 + 0,09 (N=3). The multitest score was 15,3 + 7,0 (N=85) preoperatively and 14,2 ± 6,5 (N=31), 18,0 ± 7,6 (N=15), 21,5 ± 6,6 (N=11), and 19,9 ± 5,2 (N=8) when controlled 4 times postoperatively. Tuberculin skin test reactivity increased from a score of 2,9 ± 3,0 (N=85) preoperatively to 3,5 ± 3,7 (N=31), 3,7 ± 3,8 (N=15), 5,6 ± 2,9 (N=11), and 4,8 ± 2,3 (N=8) postoperatively. Scores of skin reactivity were not correlated with TNM grading or T-lymphocyte subsets. Tumor patients with renal cell carcinoma had abnormal cellular immunity and it seems that cellular immunity improves after tumor resection.

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PHYSIOLOGICAL AND PATHOLOGICAL ALTERATIONS IN THE PROSTATIC POLYAMINES

K. Purvis, H. Rui, H. Jacobsen and K.J. Tveter

The levels of putrescine and the polyamines spermidine and spermine were analysed by high performance liquid chromatography in seminal plasma, urine and prostatic tissue under a variety of physiological and pathological situations. Their prostatic origin was confirmed by split ejaculates and by the absence of alterations in their concentrations after vasectomy. Frequent ejaculation caused alterations in the concentration of the polyamines in a direction which was opposite to other prostatic secretory parameters. The ejaculates of middle-aged men (50-55 years) contained significantly lower levels of putrescine but normal levels of spermidine and spermine when compared to young men (20-25 years). Analysis of the urine from men with normal, hyperplastic and cancerous prostates revealed major differences in the levels of the polyamines, the significance of which will be discussed.

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IMMUNOHISTOCHEMICAL DEMONSTRATION OF ESTRAMUSTINE BINDING PROTEIN (EMBP) IN THE PROSTATE OF RAT AND MAN.

S.H.Flüchter, H.J.Nelde, K.Peter, K.-H.Bichler

The antimitotic agent estramustinephosphate is used in the therapy of prostatic cancer because of its high binding to EMBP. The protein can be stained and demonstrated using a modified PAP technique. We confirmed the androgen dependance of this protein proven by Aumüller, Björk, Höghberg and Pousette in animal experiments. In rats a loss of EMBP staining occurs in glandular tissue after castration. Human prostates showed an immune reaction like rat prostate. EMBP could be detected only in epithelial cells and glandular lumina. The immunohistochemical behaviour of EMBP was studied in tissues of BPH (n=40), untreated PC (n=50) and metastasising PC (n=40) with various forms of hormonal therapy. No correlation between the patient's age or BPH size and the EMBP content was found. EMBP was seen in all untreated PC tissue samples in different concentrations. There was no correlation between staining and grade of malignancy. Two staining patterns of EMBP could be differentiated: A diffuse and an irregular focal staining. Whether the focal EMBP pattern can be interpreted according to Isaac's theory of heterogeneous clones or only as a different secretion phase has yet to be determined. PC-tissue in remission under hormonal therapy resulted in a loss or at least distinct diminution of staining. PC in hormone relapse showed a new massive EMBP reaction. The phenomenon why despite persistent androgen deprivation this cancer tissue showed a new massive EMBP synthesis is not yet clarified

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FURTHER OBSERVATIONS ON THE CITRAL-INDUCED EXPERIMENTAL MODEL OF BENIGN PROSTATIC HYPERPLASIA (BPH) IN THE RAT

C. Servadio and A. Abramovich

In a previous communication, we reported our initial observations of BPH of the ventral lobe induced by the topical daily application of CITRAL (3,7 Dimethyl-2,6-octadienal) in 42 day-old Wistar rats. These changes occurred within a few weeks.

Further studies have confirmed such unique findings. Histological and endocrinological observations have been collected since, which contribute extremely interesting data. Similar experiments performed under special conditions have induced early malignant changes together with BPH. Although the exact mechanism by which CITRAL induces such changes is as yet unclear, the experimental model in itself appears to be extremely promising and of great value for the study of the possible role of the various mechanisms involved in the development of Benign Hyperplasia and also of cancer.

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SEMINOMA IN IRELAND (1980-85): A NATIONWIDE REVIEW

J.A. Thornhill, J.J. Fennelly, D.G. Kelly, A. Walsh and J.M. Fitzpatrick.

All cases of seminoma in Ireland between 1980-'85 were analysed (n=101). A 94% three year survival in Stage 1 was reduced to 80% overall by the poorer prognosis in metastatic disease (Stage 11=72%. Stages 111+IV=23%) and the high proportion of such patients in this series (37%). Adjuvant radiotherapy, the mainstay of treatment (70%), achieved excellent remission rates (Stage 1=94%, Stages 11A+11B=83%). A surveillance only policy was effective in Stage 1 (23% recurrence, 100% ultimately alive and well), but its widespread introduction was not supported in the current context. Radiotherapy failed to achieve satisfactory disease control in advanced disease (relapse Stage 11C=29%, 111+1V=100%). Combination chemotherapy was more effective (remission rate Stage 11C=100% 111+1V=60%), but benefits in early Stage 11 were less conclusive. Disease stage was the crucial prognostic factor (p=0.0001), but advanced age, anaplastic pathology and incomplete primary surgery also conferred higher risk. The suboptimal results with metastases, highlight the need for early presentation, prompt diagnosis, precise staging and definitive management strategies.

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CYTOLOGICAL AND ENDOCRINOLOGICAL INDEX IN PROSTATIC CANCER: PROSPECTS AND LIMITS.

F. Di Silverio, G. Concolino, R. De Vita, F. Sciarra, A. De Matteis and R. Tenaglia.

The surgical staging of prostatic cancer is essential for a rational approach to management. Biological staging of the disease can be obtained from cytological and endocrinological data. Flow cytometry and grade have been used for a cytological index (CI) and hormone receptors (Ac) plus tissue DHT have been used for an endocrine index (EI).

Three groups of patients have been identified for each index - favourable, intermediate and adverse:-

Table 1.

1. G1	Diploid	4/4 (100%)	CI - favourable
2. G2	Diploid	7/12 (58%)	CI - intermediate
	Aneuploid	5/12 (42%)	
3. G3	Aneuploid	4/4 (100%)	CI - adverse

Table 2.

1. Receptor +,	DHT $>$1.5 ng/g	15/16 (94%)	EI - favourable
2.(Receptor +,	DHT $<$1.5 ng/g	1/16 (6%)	EI - intermed-
(Receptor -,	DHT $>$1.5 ng/g	4/7 (57%)	iate
3. Receptor -,	DHT $<$1.5 ng/g	3/7 (43%)	EI - adverse

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DOES DRUG BINDING TO THE NUCLEAR PROTEIN MATRIX LEAD TO CELL DEATH.

Elisabeth Kruse and Beryl Hartley-Asp

Estramustine [estradiol 3-N-bis(2-chloroethyl carbamate)] is an active metabolite of Estracyt®, an agent useful in the treatment of advanced prostate cancer. It has been shown to act as an antimitotic agent in the human prostatic cell lines DU 145 and PC-3 (1) but this mode of action cannot account for all the cytotoxicity. Therefore the effect of estramustine on the human prostatic tumour cell line 1013L was investigated. Cell proliferation experiments revealed that estramustine cytotoxicity varied during the different phases of cell growth. Maximum cell kill was found in early log phase but cell death also occured in the stationary phase. Mitotic arrest was found at cytotoxic concentrations throughout the log phase but not in the stationary phase. Subcellular distribution studies showed that the cellular uptake of estramustine increased throughout the log phase and remained steady during the stationary phase. Nuclear uptake in contrast was similar in all phases, whereas the preferential binding to the nuclear protein matrix was found to increase from 10 to 20 % during the stationary phase of growth.

As estramustine does not cause DNA damage this implicates the nuclear protein matrix as a target for cytotoxicity.

1. Hartley-Asp, B. The Prostate 5 93-100 (1984).

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