Symposium no. 5: Molecular Basis of Drug Resistance

5.025

ANALYSIS OF mdr 1-EXPRESSION IN HUMAN OSTEOSARCOMAS AND SOFT TISSUE TUMORS

U. STEIN*, H. KRAUSE*, J. FREGE*, P. SCHMIDT-PETER* and V. WUNDERLICH*

Intrinsic as well as acquired drug resistance is a major problem in the treatment of osteogenic and soft tissue sarcomas, as also seen in patients admitted to our clinic and treated with established protocols (including adriamycin and vincristine). To analyze the multidrug resistance (MDR) phenotype in those, not yet studied tumors, we started an examination of mdr 1-expression in this relatively rare tumors.

Good results were obtained by using Northern blot hybridization with a mdr 1-specific riboprobe, and confirmed in slot blot experiments. Positive signals with this method correlated with the degree of MDR in a control ovarian cancer cell line, and negative signals with absence of MDR. To verify the mdr 1-signals, control hybridizations with a GADPH-specific probe were carried out.

Up to now 31 specimens were included in the analysis, among them osteosarcomas (8/8 mdr 1-positive), malignant fibrous histiocytomas (5/6), malignant peripheral nerve sheath tumors (5/7) and some other soft tissue tumors (8/10).

This finding demonstrates, that a similar pattern of mdr 1-expression is displayed among this rather heterogeneous group of tumors. Further studies are required to correlate the mdr 1-expression with the clinical outcome.

Institute of Cancer Research, Departments of Carcinogenesis*, Pathology+ and Surgery*, O-1115 Berlin, FRG.

5.026

The Culture of renal carcinoma cells in vitro

MO Symes, Teresa Lai, Rachel Angus, CMP Collins*, PJB Smith
University Departments of Surgery and Pathology,* Bristol Royal Infirmary, Bristol BS2 8HW UK

Carcinoma cells were separated from 27 renal cell carcinomas by centrifugation of an enzymatic digest of the solid tumour on a Nycodenz^R column. Sixteen tumours were maintained in culture for >50 days and at least three passages. The epithelial nature of the cultured cells was confirmed by their expression of cytokeratins in 11 cases. In five of these the tumour cells co-expressed propyl 4-hydroxylase characteristic of fibroblasts, which was also seen in two tumours whose cells did not express cytokeratins. Expression of this enzyme increased in two tumours which were re-examined after an increased period of culture. Ploidy was examined in 12 tumours: eight were diploid, two tetraploid and two anuploid. Of the five diploid tumours examined all showed loss of contact inhibition so that the tumour cells formed multiple layers in culture. This was also true for the one tetraploid tumour examined and both anuploid tumours. One anuploid tumour failed to express cytokeratins on repeated examination but did express propyl 4 hydroxylase. Five of ten tumours showed colony formation in soft agar.

5.027

The effect of verapamil in potentiating the anti-tumour action of doxorubicin on renal carcinoma cells in vitro

MO Symes, Teresa Lai, CMP Collins*, AP Morgan, PJB Smith and BR Stonebridge**
University Departments of Surgery, Pathology* and Computer Science**, Bristol Royal Infirmary, Bristol BS2 8HW

Cells from 19 renal carcinomas were established in culture and 11 showed loss of contact inhibition when the cells became confluent. Ploidy was determined for 11 tumours: eight were diploid, one tetraploid and two anuploid. When first tested a concentration of 1μg/ml doxorubicin produced >75% inhibition of protein synthesis in vitro by carcinoma cells from 12 tumours, as measured using a [75 Se] Selenomethionine uptake essay. At a 3.3μM concentration racaemic verapamil potentiated the action of doxorubicin by a factor of >2 in 5/10 of the tumours. However, a similar enhancement of drug sensitivity was seen in 7/7 more resistant tumours. Initial sensitivity to doxorubicin and its enhancement by verapamil was not related to either ploidy of the tumour cells or to their expression of glycoprotein P170 as determined by reactivity with monoclonal antibody C-219.

5.028

MOLECULAR BASES OF MULTIDRUG RESISTANCE IN HUMAN TUMORS

Toffoli G., Viel A., Tumiotto L., Giannini F. and Boiocchi M.
Experimental Oncology 1, Centro di Rif.Oncologico, Aviano, (PN) ITALY.

MDR1, GST-π and topoisomerase II gene expression have been investigated in 150 primary human carcinomas (colon carcinomas, renal cell carcinomas, ovarian cancers, lymphomas, head and neck cancers) and 45 drug resistant human colon cell lines. These latter were selected in our laboratory with drug involved (doxorubicin and VM-26) or not (methotrexate) in the MDR phenotype. Overall analysis of experimental and clinical data indicated that MDR in human cancers is a multifactorial phenomenon determined by different biochemical mechanisms. In this phenomenon MDR1 gene product seems to have a major role. Topoisomerase appears to have weak implications, whereas GST-π seems to be related to the neoplastic transformation process more than to drug resistance. Experimental observations indicated that drug extrusion out of the cells is not the principal biochemical mechanisms with which MDR1 gene products confer to human tumor the MDR phenotype. Infact, in the range of MDR1 gene expression observed in human tumors, MDR1 seems to act principally at intracellular level by affecting distribution of the drugs and preventing them to reach the target sites of their cytotoxic effect.

5.029

Acquired resistance to cisplatin in human in vitro cell lines correlates with resistance to photons and neutrons. Intrinsic resistance does not.

Hilmar M Warenius, Richard A Britten, Peter Twentyman* and John Masters#.
CRC Oncology Research Unit, Dept of Medicine, University of Liverpool, *MRC Clinical Oncology and Radiotherapeutics Unit, Hills Road, Cambridge, # Institute of Urology, St Paul's Hospital, London.

We have studied the relative response to cisplatin, low LET x-rays and 62.5 MeV (p-Be) fast neutrons of 10 human in vitro cell lines, 5 of which have also been selected for acquired resistance to cisplatin.

Cell survival curves following exposure to cisplatin were exponential for both the innately sensitive parent lines and the variants selected for acquired cisplatin resistance, indicating that cisplatin exposure was inducing a resistance mechanism rather than selecting a resistant subpopulation within the parent cells.

No correlation between intrinsic resistance to cisplatin and either radiation modality was observed. By contrast all 5 cell lines with acquired resistance to cisplatin were resistant to fast neutrons. Cross resistance of these lines to photons was variable being most marked in the most photon sensitive cells.

We conclude that there may be molecular mechanisms of resistance to cisplatin and fast neutrons not shared by photons but question whether cell lines artificially selected in the laboratory are the most appropriate models of the clinical situation.

5.030

VERAPAMIL DOES NOT INTERACT WITH CISPLATIN IN TREATMENT OF XENOGRAFTED SQUAMOUS CELL CARCINOMA.

J Wennerberg, R Rydell, E Kjellén, Depts of ORL and Oncology, University Hospital, Lund, Sweden.

Verapamil (VPM) and other Ca-channel blockers can antagonise the multidrug resistance (MDR) fenotype. Cisplatin (CDDP) is not associated with the MDR fenotype, still VPM enhance the effect of CDDP on xenografted neuroblastoma. Since this type of biochemical modulation is very specific for tumour type we tested the hypotesis on squamous cell carcinoma (SCC) of the H&N, a tumour type commonly treated with CDDP.

Materials & Methods: Two xenografted SCC lines were tested and three different administration schedules were used; A/ 12.5 mg VPM/kg 30 min before 5.0 mg CDDP/kg; B/ 8.3 mg VPM/kg 30 min before and 24 and 48 hr after 5.0 mg CDDP/kg; C/ 12.5 mg VPM/kg 30 min before 1.7 mg CDDP/kg every second day x 3. All drugs were given i.p. Physiol. saline were given as control. Specific growth delay and area under the growth curve (AUC) were used as end-points.

Results: The mortality was low and randomly distributed. VPM alone was without effect on tumour growth. VPM did not enhance growth retardation induced by CDDP.

Conclusion: The interaction between VPM and CDDP seems to be very specific with respect to tumour type. Thus, though VPM and CDDP do not interact in treatment of SCC, this specificity does not rule out the possibility of enhancement of CDDP effect on SCC by other classes of ca-channel blockers.