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CHEMOSENSITIVITY TESTING IN PROLIFERATING MONOLAYER CELL CULTURES OF MALIGNANT TUMORS

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The choice of carcinostatic drugs for clinical chemotherapy depends largely on empirical data without certainty that the drug selected will inhibit growth of a given tumor. In our study an assay system for predictive drug testing was used which is based on the proliferation of cultures of freshly explanted tumor cells.
Cell characterization: To ensure the malignant character of the cultured cells nuclear DNA was quantified by cytophotometry showing hypertetraploidy. By immunocytochemistry (IH) cytokeratin was stained in epithelial, vimentin in mesenchymal cells. Tumor markers (CEA, CA 125, CA 19-9) were found in accordance with the original tumor.
Proliferation assay for drug sensitivity: To evaluate dose-effects 3 clinically relevant drug concentrations were tested during logarithmic growth of cell strains derived from 25 intestinal carcinomas, 15 sarcomas, 10 miscellaneous tumors. Effectiveness of the drugs was deduced from the % inhibition of cell growth in relation to untreated controls. Depending on the drugs applied the cell strains showed individual reaction pattern. The in vitro results correlated positively with the clinical follow-up in more than 80%.
IH and DNA cytophotometry were used to elucidate (1) epithelial or mesenchymal origin, and (2) the malignant character of the cultured cells. The usefulness of our in vitro assay to predict chemosensitivity in vivo was confirmed by the clinical results. The data show the uniqueness of each tumor requiring individualized systemic therapy.

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THE COLONY ASSAY IN-VITRO AS A PROCEDURE TO REDUCE ANIMAL TESTING IN THE DEVELOPMENT OF NEW ANTICANCER DRUGS.

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Over the past 40 years, new compounds have been tested for potential anticancer activity in transplantable tumors in mice and rats. The number of animals needed for this purpose has been considerable. Recently a public movement has demanded the discontinuance of animal experimentation due to ethical reasons. In the past years, we replaced in-vivo testing of new compounds in nude mice by in-vitro experiments in cell culture. After several cell divisions single tumor cells develop into tumor colonies.This so-called colony assay has shown a good correlation to the tumor response in clinical studies. Using a modification of the Hamburger & Salmon assay, 117/136 (86%) tumors growing subcutaneously in nude mice grew ($\geq$ 30 colonies).
The significance of the colony assay lies in the development of new drugs against cancer. New compounds are first tested in 20 selected human tumors, in the mouse leukemia P388 as well as in human bone-marrow. A critical point is determining the relevant dose level for the clinical situation. For this reason, the 2 most responsive tumors are additionally investigated in-vivo. Using these techniques the effects of several new drugs will be demonstrated. Due to the development of the colony assay, animal experimentation now comprises less than 10% of all therapeutic experiments in our laboratory. Furthermore, target tumors for clinical phase I and II studies can be determined.
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DOSE RESPONSE EFFECTS OF ADRIAMYCIN, CISPLATIN, MITOMYCIN-C, 5-FLUORURACIL, VEPESID AND VINDESIN ON HUMAN TUMOR XENOGRAFTS IN THE COLONY ASSAY.

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In order to use the colony assay in-vitro for pretherapeutic drug testing of tumors, the relevant dose level for clinical activity has to be determined. Using continuous drug exposure and a modification of the Hamburger & Salmon assay we studied the colony inhibition of 6 clinically used agents over a wide dose range. A drug was considered to be effective if a colony inhibition of less than 30% of the control was found. The reproducibility of the in-vitro results could be confirmed. For each compound there was a clear relationship between the rate of responsive tumors and drug dosage. Comparison to clinical response rates in responsive tumor types was used to determine the relevant dose level in-vitro. Response rates for the 6 drugs at the relevant dosages are shown in the table.

	Dose* µg/ml	Effective** / Total	
Adriamycin	0.03	19 / 45	42%
Cisplatin	0.1	15 / 46	33%
Mitomycin-C	0.005	12 / 37	27%
5-Fluoruracil	0.2	7 / 22	32%
Vepesid	0.3	10 / 28	36%
Vindesine	0.01	12 / 40	30%

* Continuous exposure; ** Test/Control $\leq$ 30%

These dosages are used to test the drug sensitivity of newly established tumors in the nude mouse system as well as of tumor biopsies taken directly from the patients.

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BLOOD FLOW, OXYGEN CONSUMPTION AND SUBSTRATE UTILIZATION OF HUMAN TUMORS XENOTRANSPLANTED INTO NUDE RATS

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A new model has been developed which allows for the first time the systematic study of tumor blood flow (TBF), of the O_2 consumption and of the substrate utilization of human tumor tissue in vivo under controlled systemic conditions.
Material and Methods: Human mammary and ovarian carcinomas xenotransplanted into thymusaplastic, T- cell- deficient rnu/rnu rats were investigated. An epigastric pouching technique was used to obtain a tissue- isolated tumor in the inguinal region, connected to the host by one artery and one vein. Pouches with fat and/or granulomatous tissue served as controls. During Pentobarbital- anaesthesia, TBF was measured by a direct venous outflow technique. O_2 consumption, glucose uptake, lactate release and the turnover of ß- hydroxybutyrate, acetoacetate, free fatty acids and amino acids as well as the detoxification of ammonia and urea were evaluated from TBF and the respective arteriotumorvenous concentration differences.
Results: TBF decreases with increasing tumor wet weight in breast cancers as well as in the ovarian tumors investigated. A similar weight- related decrease is also observed for O_2 and glucose consumption rates in the xenotransplants, indicating that the nutritive blood flow is the main determinant of the consumption rates, irrespective of tumor type and histology. All tumors release lactate, the release rate being linearly dependent on the glucose consumption rate. The ketone bodies are taken up on the average by all the tumor types investigated. There is a net release of most amino acids by the xenotransplants. However, no single amino acid is consistently released or taken up. The fatty acids are on the whole released by the breast cancer tissue, probably indicating de novo synthesis. So far, in the ovarian carcinomas no definite pattern is seen for the handling of free fatty acids.

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