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THE INFLUENCE OF 5-FLUOROURACIL ON THE APPEARANCE OF ENDOTHELIUM IN SMALL ARTERIES. A SCANNING AND TRANSMISSION ELECTRON MICROSCOPIC STUDY IN RABBITS

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Cardiotoxicity of 5-fluorouracil (5-FU) is mysterious toxic manifestation in treatment of human malignancies. Its possible mechanism might be direct cytotoxic effect on endothelium in cardiac vessels. We tested this hypothesis in experimental study in rabbits. The small arteries after *in vivo* treatment with 5-FU were prepared by perfusion-fixation method. Scanning and transmission electron microscopy evaluation of endothelium was done. We found irreversible cell damage consisting of disruption of endothelium sheet and patchy exposure of subendothelium, frequently observed as location for thrombus formation. Findings support the hypothesis about the thrombogenic effect of 5-FU, secondary to its direct cytotoxic effect on endothelium as pathophysiological mechanism behind 5-FU cardiotoxicity.

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THE PHARMACOKINETICS OF TOPOTECAN (T) AND ITS CARBOXYLATE (C) FORM FOLLOWING SEPARATE INTRAVENOUS ADMINISTRATION TO THE DOG

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In vitro, T undergoes a reversible, pH-dependent hydrolysis of the active lactone form to C. To study the kinetics of this reaction *in vivo*, T and C were separately administered as 30 minute intravenous infusions to female Beagle dogs in a cross-over design. T was also administered orally to the same dogs. After intravenous dosing, T underwent interconversion to C and vice versa. Clearance of T from the body was faster than interconversion to C but clearance of C was slower than interconversion to T. The Vss of T was approximately 9-fold greater than Vss of C. Following oral administration of T, the bioavailability was approximately 50% whether conventional or equations incorporating reversibility were used. After iv administration of T, the amount of T in the dog was much greater than that of C even though their respective plasma concentrations were similar.

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EFFECTS OF KP 735, A NEW PLATINUM-BASED ANTITUMOR AGENT ON CLONOGENIC GROWTH OF FRESHLY EXPLANTED HUMAN TUMORS *IN VITRO*

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Cis-Diammine[(bis(phosphonatomethyl)amino)acetato(2−)−O^1,N^1] platinum (II), (KP 735) is a new platinum-based agent which has shown antitumor activity in established tumor cell lines. We have studied the effects of KP 735 on soft agar colony formation of freshly explanted human tumors *in vitro*. Fifty-three out of 74 specimens (72%) are evaluable. Major tumor subgroups were: breast (9), mesothelioma (7), melanoma (6), kidney (5), carcinoma of unknown primary (6). Using a continuous incubation (21–28 days), concentration-dependent inhibition (colony formation $\leqslant 0.5 \times$ control) was observed with 0/53 specimens inhibited at 0.1 μg/ml, 9/53 (17%) at 1.0 μg/ml and 32/53 (60%) at 10 μg/ml. In a short-term incubation (1 hour), concentration-dependent inhibition was observed with 1/51 specimens (2%) inhibited at 0.1 μg/ml, 3/51 (6%) at 0.4 μg/ml, 6/51 (12%) at 1.0 μg/ml, 15/51 (29%) at 10 μg/ml and 30/51 (59%) at 100 μg/ml. At the highest concentration, KP 735 was more active than vinblastine (12/33 specimens (36%) inhibited, $P = 0.043$ McNemar's test), cisplatin (16/51 (31%), $P = 0.001$) and carboplatin (11/51 (22%), $P = 0.001$). We conclude that KP 735 has antineoplastic activity against freshly explanted human tumor cells *in vitro*. The spectrum of activity includes tumors with known clinical resistance to conventional chemotherapy. Further clinical development of this agent thus seems warranted.

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EFFECTS OF KP 1220, A NEW PLATINUM COMPOUND, ON COLONY FORMING UNITS FROM FRESHLY EXPLANTED HUMAN TUMORS *IN VITRO*

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Dichloro(5, 7, 8, 10, 11, 13, 14, 16-octahydro [1, 4, 7, 10] tetraoxacyclohexadecino- [13, 12-b: 14, 15-b′] dipyridine-N^1, N^{20}) platinum (II), (KP 1220) is a platinum analog reacting with nucleophilic sites on DNA, causing intra- and interstrand crosslinks as well as DNA-protein crosslinks. This agent has shown antitumor activity in established tumor cell lines. We have studied the effect of KP1220 on *in vitro* soft agar colony formation of 77 freshly obtained human tumors using a capillary cloning system. Major tumor subgroups were: 13 melanoma, 10 mesothelioma, 9 renal, 7 breast, 6 non-small cell lung, 6 ovary, and 6 carcinoma of unknown primary. Final concentrations were 0.1, 1.0, 10.0 μg/ml for continuous exposure (21–28 days) and 0.1, 0.4 (equimolar to cisplatin), 0.48 (equimolar to carboplatin), 1.0, 10.0 μg/ml for short-term (1 hour) exposure. Using a continuous exposure, 52/77 specimens (68%) showed evaluable colony formation in controls. KP 1220 inhibited tumor colony formation in a concentration-dependent manner with 2/52 specimens (4%) inhibited at 0.1 μg/ml, 12/52 (23%) at 1.0. μg/ml and 36/52 (69%) at 10.0 μg/ml. Using a short-term exposure, 49/77 specimens (64%) were evaluable. Again, concentration-dependent inhibition of tumor colony formation was observed with 1/47 specimens (2%) inhibited at 0.1 μg/ml, 9/49 (18%) at 0.4 μg/ml, 16/48 (33%) at 1.0 μg/ml and 35/49 (71%) at 10 μg/ml. At concentrations $\geqslant 1$ μg/ml, KP 1220 was as active as other clinically used antineoplastic agents. We conclude that KP 1220 has antitumor activity. Further clinical development of these agents should be considered.

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EVALUATION OF TOPOTECAN AGAINST THE SK-MES HUMAN LUNG CARCINOMA XENOGRAFT

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Topotecan, a promising topoisomerase I inhibitor being developed as an anticancer agent, was evaluated vs the SK-MES human lung tumor xenograft in nude mice. Topotecan (4 mg/kg; daily × 5) was tested as a single agent, and in combination with navelbine or gemcitabine. Topotecan alone was quite effective vs SK-MES, causing 75% tumor growth inhibition, with greater than 50% tumor shrinkage in several mice. Neither gemcitabine (3 mg/kg; daily × 5) nor navelbine (2 mg/kg; daily × 5) as a single agent was active in this model. Addition of gemcitabine or navelbine to the topotecan regimen did not improve the efficacy of topotecan given alone. Also, each combination resulted in a number of drug-related deaths in mice, without improving the efficacy of topotecan. In conclusion, topotecan demonstrated excellent activity as a single agent against the SK-MES human lung tumor xenograft. The data suggest that topotecan may have potential as an antineoplastic agent in patients with lung cancer.

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EXPRESSION OF LUNG RESISTANCE-RELATED PROTEIN (LRP) IN LUNG CANCER

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Expression of LRP seems to be of prognostic relevance in advanced ovarian cancer and acute myeloid leukemia. In this study we evaluated the expression of LRP in lung tumor samples derived from patients and its relation to survival, tumor differentiation, TNM classification and histology. Immunohistochemical staining (IH) was performed on 42 frozen lung tumor tissue samples using the mouse monoclonal antibody LRP-56 (IgG2b) directed to the 110 kD protein. LRP expression was calculated as the percentage of tumor cells which stained with the antibody. As cut-off point for Kaplan-Meier curves we took 10% LRP expression; comparisons of survival curves were performed by Mantel-Cox

analysis. Kruskal-Wallis one-way nonparametric AOV was used to estimate whether the LRP expression was related to: tumor differentiation (well, moderately, poorly); TNM classification and histology. IH of the tumor sections showed different expression of LRP in the different histological subtypes of lung cancer (squamous cell carcinoma 83%; adenocarcinoma 59%; large cell/undifferentiated carcinoma 36% and SCLC 5%). LRP expression was significantly higher in squamous cell carcinoma than in the other subtypes ($P < 0.05$). Furthermore adenocarcinomas showed a significant ($P < 0.05$) higher LRP expression than the SCLC. No significant difference in expression levels was found between patients with different TNM-classification or tumor differentiation. In this relatively small group of patients, there was no relation between LRP expression and survival. Prospective research is being performed in patients undergoing chemotherapy.

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IDOXIFENE DELAYS ACQUIRED ANTI-OESTROGEN RESISTANCE IN MCF-7 HUMAN BREAST CANCER XENOGRAFTS

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Idoxifene (IDOX) is a new anti-oestrogen with less agonist activity than tamoxifen (TAM). We (i) compared the inhibition by IDOX and TAM of the growth of MCF-7 breast cancer xonografts, (ii) determined whether IDOX delayed acquired resistance, and (iii) assessed whether IDOX inhibited the growth of TAM resistant tumours. Forty tumours were established with oestradiol (E2) support in ovariectomised athymic mice and continued with E2, no support, TAM or IDOX (mean serum TAM 35 ng/ml, IDOX 28 ng/ml). The reductions in tumour volume (mean percentage baseline ± SEM) after 2 and 6 m were as follows: TAM 71.8% (± 10.5) and 81.1% (± 14.8); IDOX 47.2% (± 9.3) and 51.0% (± 14.3); E2 withdrawal 30.7% (± 5.2) and 13.1% (± 4.3), respectively. IDOX appeared to give greater tumour regression compared with TAM. Furthermore, after 6 months 3/10 TAM-treated but 0/10 IDOX-treated tumours developed acquired resistance and started to re-grow. In separate studies significantly fewer TAM-resistant tumours were supported by IDOX than by TAM (3/12 vs 8/12; $P = 0.04$ Chi-Squared). These data indicate that IDOX shows reduced growth support of MCF-7 xenografts compared with TAM and appears to delay the development of acquired resistance. Furthermore IDOX may share only partial cross-resistance with TAM. The reduced agonist activity of IDOX may, in part, explain these observations.

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MARINE (MA) DEPSIPEPTIDES (DEP) WITH ACTIVITY (A) AGAINST SOLID TUMOURS (ST) MODELS

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Two MADEP from our R + D program are presented. KahalalideF (KF) is a MADEP isolated from a Hawaiian mollusc; it displays selective A *in vitro* (IVT) in Prostatic cancer cell lines (PROca) (TG1 at 4.22E−07 MOLAR), COMPARE analysts (COa) fails to match KF with any standard anticancer drug and initial data indicates KF promotes an extensive vaculorization in cultures COS1 and HELA cells and might interact with specific component(s) of the cell surface. *In vivo*, KF lacks A in P388 but has A in A549 lung ca xenograft (X) (37% T/C at KF dose = 2 mg/kg/Q4D × 3). Thiocoraline (THIO), MADEP isolated from a MA micro-organism from Mozambique; THIO has IVT A in melanoma, colon and lung ca cell lines (TGIs = 4.09E-09, 2.50E-09 and 2.50E-09 respectively). THIO binds to DNA (> luM); kinetic studies suggests THIO inhibits cell cycle progression at G1, S, G2 and M phases (reversible after washing) and COs matches THIO with doxo and daunorubicin. THIO lacks *in vivo* A in P388 but has A in A549 X (31%T/C; 6 mg/Kg/Q4Dx3). THIO assessment in NSCLC and colon Xs is ongoing. Large scale supply is feasible by fermentation.

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ACTIVITY OF N4-OCTADECYL-ARA-C IN HUMAN SOLID TUMOR XENOGRAFTS AND LEUKEMIAS

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A series of new Ara-C derivatives with alkyl chains at N4 has been tested in-vivo in subcutaneously growing human xenografts. The liposomal preparation of N4-Octadecyl-Ara-C (NOAC) was studied in 2 human leukemias and in 6 solid cancers. At the MTD of 150 mg/kg/day injected ip on day 1, 4, 7 and 10 NOAC showed a higher antitumor activity than the equitoxic dose of Ara-C in the promyelocytic leukemia HL-60, tumor volumes being 3% and 52% of the controls, respectively. In the acute T lymphoblastic leukemia CCRF-CEM both compounds were highly active. An impressive *in vivo* activity could be demonstrated in solid tumors. In the mammary cancer MAXF 401 NOAC effected a T/C of 18% versus 42% obtained with Ara-C, in the small cell lung cancer LXFS 605 T/C values were 27% versus 56%. The new derivative showed activity in a large cell cancer of the lung and in the prostate cancer PC3M with a T/C of 17%. The activity in PC3M was higher than obtained with 7 standard agents. In conclusion the new Ara-C derivative NOAC is a promising new compound which should be developed in solid tumors (mammary and prostate cancers) as well as in leukemias.

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ADDITION OF OXALIPLATIN (L-OHP®) TO CHRONOMODULATED (CM) 5-FLUOROURACIL (5-FU) AND FOLINIC ACID (FA) FOR REVERSAL OF ACQUIRED CHEMORESISTANCE IN PATIENTS WITH ADVANCED COLORECTAL CANCER (ACC)

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L-OHP®/5-FU/FA CM combination partially circumvented 5-FU resistance in patients (pts) with ACC (*Cancer* 1992, **69**, 893). L-OHP® delivered as q 3 wks CM or bolus showed a 10% (14/138) overall response rate (ORR) in ACC pts with proven progressive disease (PD) while getting 5-FU/FA treatment. The non-cross resistance and/or synergy of L-OHP® with 5FU/FA was assessed in 25 pts with acquired resistance (20 with CT scan-proven [PD]—and 5 with median 5 months [2.5–12] disease stabilisation [ST]) while on 5-day (d) CM 5-FU (700–1000 mg/sqm/d)/FA (300 mg/sqm/d) (peak delivery at 4.00 h) (FF). L-OHP® (20 to 25 mg/sqm/d, peak at 16.00 h) was added to this schedule in 2nd (12 pts) or 3rd line (13 pts) (FFL) according to 2 different schedules: 5d q 3 wks (14 pts) or 4 d q 2 wks (11 pts). *Selection criteria:* Pretreated ACC, no other intercurrent chemotherapy between the 2 schedules, measurable lesion. *PT Characteristics:* M/F = 10/15, median age = 59, colon/rectum = 14/11, PS 0–1 vs 2–3 = 22/3, nb of sites <2 vs ⩾ 2 = 8/17, liver involvement = 21 (94%). Previous 5-FU time exposition = median 7.2 months (1.5–25.2), FF (CM) median dose intensity (DI) = 1050 mg/sqm/wk. *FFL (CM) Treatment:* 171 cycles, median = 7 (1–15), time to exposition = 5 mo (1–9), 5-FU DI = median 1072 mg (831–1580), L-OHP® DI = median 35.7 mg (24–43). *Toxicity (per pt):* grade 3–4: nausea-vomiting = 25%, diarrhea = 16%, mucositis = 8%. No gr 3–4 hematotoxicity. No renal or auditive toxicity was observed. No toxic death. *Efficacy:* 7 PR (29.2%), 5 minor reponses (21%), 4 SD (17%) and 8 PD (33%). One pt too early. Duration of response was 8.5 mo, disease-free progression 5.8 mo and median survival 12 mo. *Conclusion:* Addition of L-OHP® can reversed FF resistance to CM 5-FU/FA in one third of pts. This further suggests a synergistic and/or modulatory effect between 5-FU and L-OHP®.

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DRUG RESISTANCE MECHANISMS TO CISPLATIN IN H-HAS AND V-MYC TRANSFECTED FIBROBLASTS

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Cisplatin is a widely used antitumor agent. To investigate the role of oncogenes in drug resistance to cisplatin we determined the sensitivity