Methods and Results: The maximum tolerated dose (MTD) for MTX and MTX-albumin was determined (2 mg/kg based on MTX injected on day 1, 3, and 7). 100 SD-rats bearing Walker-256 tumors received injections of the MTD and of MTD/2 of MTX, MTX-albumin and of mixtures containing MTD/2 or MTD/4 of both MTX and MTX-albumin (MTX-MIX). In addition 30 Copenhagen rats bearing a MTX resistant slowly growing Dunning Hi prostate adenocarcinoma (Du HI) were treated with the MTD of MTX and of MTX-albumin. No side effects were observed. MTX-albumin conjugates were more effective than MTX alone in terms of growth retardation of the Du Hi tumor ($p < 0.001$). In the Walker-256 tumor bearing rats, cures and growth retardation were observed with the lowest rates for MTX alone, than for MTX-albumin, and with the best results for the combination of MTX and MTX-albumin. This was confirmed for the MTD and MTD/2 group. At 1 mg/kg MTX cured 2 out of 10 rats, MTX-albumin 3 of 10, whereas a mixture 0.5 mg/kg of MTX and of 0.5 mg/kg MTX-albumin cured 6 out of 10 rats and prolonged the surviving time from 4.7 days to 7.3 days compared to MTX.

Conclusion: MTX-albumin conjugates show therapeutic activity in vivo. In combination with MTX additive effects were observed. MTX-albumin conjugates performed significantly better than the parent compound in a slow growing rodent tumor.

801 POSTER

The antihypercalcemic action of gallium-nitrate is not due to inhibition of parathormone or parathormone-related protein secretion in rats

T. Schilling[1], H. Ritzel[2], O. Kratz[1], D.C. Gey[1], G. Delling[2], F. Raue[3], R. Ziegler[1]. [1]*Dep. of Internal Medicine I, University of Heidelberg;* [3]*Endocrine Practice, Heidelberg,* [2]*Dep. of Bone Pathology, University of Hamburg, Germany*

Purpose: Gallium nitrate (GaN), the anhydrate salt of the naturally occurring heavy metal, is able to reduce hypercalcemia of malignancy in vivo. Furthermore, GaN is able to reduce parathormone (PTH) secretion in parathyroid cells in vitro. We tested whether GaN is able to reduce PTH or parathormone-related protein (PTHrP) secretion in vivo.

Methods: We used female Fisher rats, weighing 160–200 g. Humoral hypercalcemia of malignancy was induced by subcutaneous inoculation of 10^6 Walker carcinosarcoma (WCS) 256 cells. PTH secretion was examined in normal animals, after inducing hypocalcemia with 100 mg/kg EDTA intraperitoneally.

Results: 40 mg/kg GaN led to a significant reduction of WCS tumor growth (5.1 ± 1.8 vs. 7.4 ± 2.2 g) and hypercalcemia (3.6 ± 0.5 vs. 4.3 ± 0.6 mmol/l) at day 8.40 mg/kg GaN did not influence PTHrP serum levels in WCS bearing rats at day 8 (27.8 ± 11.5 vs. 25.9 ± 6.2 pmol/l), whereas osteoclast surface (OcS/BS) was significantly reduced (3.5 ± 1.3 vs. $6.2 \pm 2.3\%$). EDTA-stimulated induction of PTH secretion in normal rats was not significantly reduced by 40 mg/kg GaN (133.8 ± 45.1 vs. 136.6 ± 66.8 pg/ml).

Conclusion: The antihypercalcemic effect of GaN is due to osteoclast inhibition and is not due to inhibition of PTHrP or PTH secretion in vivo.

802 POSTER

Stealth liposome entrapped doxorubicin (SLED) and cisplatin (SLEC) versus head and neck xenograft tumours

K.J. Harrington[2], G Rowlinson-Busza[1], P. Uster[3], J.S.W. Stewart[2]. [1]*ICRF Clinical Research Laboratory, Royal Postgraduate Medical School, Hammersmith Hospital 150 Du Cane Road, London;* [2]*Department of Clinical Oncology, Royal Postgraduate Medical School, Hammersmith Hospital 150 Du Cane Road, London, UK;* [3]*SEQUUS Pharmaceuticals Inc., Menlo Park, CA, USA*

Purpose: To study the effect of SLED and SLEC, compared to unentrapped doxorubicin (UD) and cisplatin (UC), in head and neck cancer xenograft tumours (HNCXT).

Materials and Methods: Groups of 8–10 nude mice with HNCXT received single i.v injections of one of the following agents: SLED, SLEC, UD or UC. Control animals received no therapy. Tumour volume was assessed on the day of treatment (Vo) and then 2–3 times per week. Mice were killed when the tumour had tripled its original volume (3Vo). Time taken to reach 3Vo was used as a surrogate measure of survival.

Results: Median times to 3Vo were as follows: 7.3 days (control); 9.3, 5.4, and 9.7 days (UD 50, 100 and 200 μg); 16.1, 18.3 and 40.6 days (SLED 50, 100 and 200 μg); 6.9 and 15.3 days (UC 100 and 250 μg); 15.9, 21.5 and 34.0 days (SLEC 100, 250 and 500 μg). Durable complete response or stable disease (>60 days) was seen after 200 μg SLED in half the mice. 500 μg UC caused the death of all animals at 5 days. No toxicity was seen with single dose SLED or SLEC.

Conclusion: SLED and SLEC show significant activity in HNCXT. Both SLED and SLEC were more active than their unentrapped counterparts. Clinical trials of both agents in patients with head and neck cancers are planned.

803 POSTER

Effects of MTA (multi-targeted antifolate, LY231514) on intracellular folate and nucleoside triphosphate pools in CCRF-CEM cells

V.J. Chen, J.R. Bewley, S.L. Andis, R.M. Schultz, C. Shih, L.G. Mendelsohn, D.E. Seitz, J.L. Tonkinson. *Lilly Research Laboratories, Indianapolis, IN, USA*

Purpose: MTA (LY231514) is a novel pyrrolo[2,3-d]pyrimidine-based antifolate, presently in phase II trials for various solid tumours. It has been shown to inhibit thymidylate synthase (TS), dihydrofolate reductase and glycinamide ribonucleotide formyltransferase *in vitro*. Both thymidine and hypoxanthine are required to completely reverse the cytotoxicity of MTA (at $\geq$30 nM) in CEM cells. The present study examined the effects of MTA on intracellular folate, ribo- and deoxyribo-nucleoside triphosphate (rNTP and dNTP) pools.

Methods: Intracellular folates were pre-labelled by culturing CCRF-CEM cells in medium containing ^{3}H-leucovorin. After drug treatment, the folates were extracted, treated with conjugase and analyzed by HPLC. Total NTPs were extracted in 60% ethanol. rNTPs were analyzed directly on HPLC, and dNTPs likewise after per-iodate degradation of rNTPs.

Results: Treatment with MTA (300 nM) for 4 h resulted in no detectable accumulation of dihydrofolate. Over 24 h, MTA caused little change in levels of rNTP, but induced a rapid loss of TTP, dCTP and dGTP (to <15%), with a concomitant rise in dATP (~30%).

Conclusion: Our data qualitatively resemble those reported for TS inhibitors (*Biochem Pharmacol* 1995; *49:* 819), suggesting that inhibiting the thymidylate cycle is a key effect of MTA in CCRF-CEM cells. Studies on the anti-purine effect of MTA are in progress.

804 POSTER

Clofazimine and B4121 sensitize an intrinsically resistant human colon cancer cell line to paclitaxel and taxotere

G. Jooné, C.E.J. van Rensburg. *MRC Unit for Inflammation and Immunity, Department of Immunology, University of Pretoria, South Africa*

Purpose: To investigate the potential of clofazimine and a more active derivative B4121, to sensitize three intrinsically resistant human colon cancer cell lines ($CaCo_2$, ATCC HTB 37; COLO 320DM, ATCC CCL 220; HT-29, ATCC HTB 38) to vinblastine, doxorubicin, daunorubicin, paclitaxel, taxotere and cisplatin at a non toxic, therapeutically relevant concentration of 0.25 μg/ml. Cyclosporin A (CsA) multidrug resistant (MDR)-modifying agent at 5 μg/ml was included for comparison.

Methods: Cell proliferation and P-glycoprotein (P-gp) expression were measured by colorimetric and flow cytometric procedures.

Results: The cell line expressing high levels of P-gp, COLO 320 DM, was susceptible to chemosensitization by the experimental agents for the P-gp substrates (paclitaxel, taxotere, daunorubicin, vinblastine and doxorubicin) but not for cisplatin. Clofazimine, B4121 and CsA increased the sensitivity of COLO 320 DM cells for paclitaxel 7, 30 and 47 fold and taxotere 5, 10 and 1460 fold respectively. $CaCO_2$ cells expressed low levels of P-gp and were only marginally susceptible to sensitization by these drugs whereas the HT-29, a P-gp negative cell line, was unaffected.

Conclusion: The riminophenazines might prove useful for inclusion in taxotere or paclitaxel chemotherapy of P-gp expressing colon cancers.

805 POSTER

Mapping drug distribution patterns in solid tumors: Toward conformal chemotherapy for local tumor control

S. Kanekal, A. Sahai, H.M. Kim, R.E. Jones, D. Brown. *Matrix Pharmaceutical, Inc., Fremont, CA, USA*

Purpose: Chemotherapeutic efficacy depends on concentration and duration of drug exposure to tumor cells. Extending our ability to map and predict local drug exposure may lead to generation of treatment algorithms such that rational conformal chemotherapy of solid tumors similar to conformal