with other analogues like trans-4-hydroxy-L-proline or cis-4-hydroxy-D-proline. The anti-tumor activity of CHP could be antagonized by the Na^+/K^+-ATPase inhibitor ouabain (1 mM) and by addition of high extracellular potassium (>70 mM), however not by the vacuolar H^+-ATPase inhibitor bafilomycin. Additionally CHP was found to decrease significantly the expression of the EGF receptor.
Conclusions: The results of the present study demonstrate that CHP effects extensive intracellular vacuolization in colon and pancreatic tumor cell lines by modulating intracellular ion concentrations of K^+, Na^+ and H^+involving Na^+/K^+-ATPase, which is differentially expressed in tumor cells. Tumor cell proliferation may be further limited by the generation of a CHP-induced acidic extracellular environment and downregulation of the EGF receptor. Therefore CHP may constitute an antitumor agent with broad activity/low toxicity targeting a unique intracellular and tumor-associated mechanism of Na^+/K^+ homeostasis.

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Reduction and activation of RH1 by NADPH cytochrome P450 reductase

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Background: RH1 is a novel diaziridinylbenzoquinone bioreductive agent that is in clinical trials. RH1 is a very efficient substrate for the two-electron reducing enzyme NAD(P)H quinone oxidoreductase 1 (NQO1; DT-diaphorase). Reduction by NQO1 results in activation of the aziridine groups and DNA alkylation with interstrand cross-link formation. Because RH1 is a very good substrate for NQO1, this agent was considered ideal for use in an enzyme-directed tumor targeting strategy to treat tumors with high levels of NQO1. However, studies have shown that RH1 can be a substrate for the one-electron reducing enzyme NADPH cytochrome P450 reductase (P450 Red), and that leukemia and lymphoma cell lines with low or absent NQO1 expression can have a high sensitivity to RH1. Thus, it is not clear if P450 Red can contribute to activation of RH1. In this study, we investigated the role of P450 Red in the reduction and activation of RH1.
Materials and methods: We compared reduction and activation of RH1 by P450 Red and NQO1. Reduction was studied by spectroscopic analysis, and production of RH1 semiquinone was monitored by EPR. DNA damage produced by reduced RH1 was measured by gel assays, and cytotoxicity of RH1 in T47D human breast cancer cells and T47D cells transfected with P450 Red (T47D-P450) was compared by MTT assays.
Results: Under hypoxia, reduction was faster with NQO1 than with P450 Red. Under aerobic conditions redox cycling was slower after reduction by NQO1 compared with P450 Red. RH1 reduction by P450 Red gave a very strong semiquinone EPR signal while NQO1 gave only a weak signal. Reduction of RH1 with NQO1 produced significantly more DNA strand breaks than reduction with P450 Red. P450 Red activity was 20-fold higher in T47D-P450 cells than in T47D cells while the levels of NQO1 were similar. Despite this, the cytotoxicity of RH1 in the two cell lines was similar. The P450 Red inhibitor, diphenyliodonium chloride (DPIC), did not inhibit RH1 cytotoxicity in T47D-P450, while the NQO1 inhibitor, dicoumarol, significantly inhibited RH1 cytotoxicity. However, if the transfected cells were treated with both inhibitors there was additional inhibition of RH1 cytotoxicity compared with dicoumarol alone. A similar study in the T47D cells showed that treatment with both enzyme inhibitors did not result in greater inhibition of cytotoxicity than treatment with dicoumarol alone.
Conclusions: These results confirm that P450 Red can reduce RH1, and in the absence of NQO1 activity high levels of P450 Red can contribute to RH1 activation and cytotoxicity. However, NQO1 appears to be the major activating enzyme for RH1, and P450 Red likely does not play a role in RH1 activation at normal cellular levels of this enzyme.
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Therapeutic benefit of combining AQ4N with radiation

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Background: AQ4N, a bioreductive drug currently in clinical evaluation, is effective at killing the hypoxic tumours cells that have a negative impact on radiation therapy. The aim of this study was to investigate the effect of combining AQ4N with radiation in a murine tumour model, and early and late responding mouse normal tissues.
Methods: Female CDF1 mice with or without a C3H mammary carcinoma in the right rear foot were used. AQ4N was dissolved in saline and intraperitoneally injected at 0.02 ml/g body weight. Radiation (240 kV X-rays) was given locally to either the right rear foot of normal or tumour bearing mice, or the lungs, of restrained non-anaesthetised animals. Response was assessed as the percentage of animals at each radiation dose showing either local tumour control at 90 days; development of moist desquamation of the foot between 11–23 days; or a 20% increase in lung ventilation rate within 9 months, after treatment. Following logit analysis of the radiation dose response curves the radiation dose producing a response in 50% of treated animals (RD50) was calculated. A Chi-squared test was used for statistical analysis ($p < 0.05$).
Results: The RD50 value (±95% confidence intervals) for control-irradiated tumours was 53 Gy (51–55). Injecting AQ4N (60 mg/kg) 0, 2 or 4 hours prior to irradiating significantly reduced the RD50 to similar values of 44 Gy (40–49), 47 Gy (44–50) and 45 Gy (42–47), respectively. Using a 2-hour interval, the respective RD50 values obtained with AQ4N doses of 30 and 120 mg/kg were 50 Gy (46–54) and 43 Gy (39–47). For skin the RD50 value for radiation alone was 32 Gy (30–33) and a small yet significant enhancement was obtained with radiation given 2 hours after injecting 60 mg/kg AQ4N; the RD50 value being 30 Gy (29–31). With lung the RD50 value was 14 Gy (11–17) regardless of whether radiation was given alone or 2-hours after AQ4N (60 mg/kg).
Conclusions: AQ4N significantly enhanced the radiation response of this C3H mammary carcinoma and did so in a dose dependent, yet time independent, fashion. Using a clinically relevant 60 mg/kg dose and a 2-hour interval gave rise to a 1.12 fold enhancement of radiation response. In early responding skin the enhancement was only 1.06 and in late responding lung absolutely no enhancement was found, resulting in a clear therapeutic benefit.
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A Phase I study with Fosfluridine tidoxil, a novel oral fluoropyrimidine, in patients with advanced solid tumors

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Methods: Sequentially, two dosing schedules were explored: 1. p.o. once qd for 14 days q 3 wks and 2. p.o. once qd for 7 days q 2 wks. For PK assessment, a single dose was given to all pts 7 d before daily dosing. An adapted Fibonacci '3+3' dose escalation design was used. 23 pts (14 women/9 men, med age 61 yrs, range 37–80 yrs) were treated with schedule 1, 19 (9 women/10 men, med age 62 yrs, range 41–73 yrs) with schedule 2. Standard Phase I eligibility criteria were required for study entry. DLT observed during the first 2 cycles with schedule 1 and the first three cycles with schedule 2 were taken into account for MTD assessment.
Results Schedule 1: Explored DL's: 50, 100, 200 and 300 mg with 3 (8), 6 (35), 10 (27) and 4 (4) pts (cycles) per DL. MTD: 200 mg with gastrointestinal (GI) DLT (diarrhea, abdominal pain, colitis, vomiting, nausea) at the end of or shortly after the first 14 treatment days in 5/12 pts (42%) and 5/27 cycles (19%). Several pts were dose-reduced due to toxicity. No DLT was observed at 100 mg.
Results Schedule 2: Explored DL's: 100, 150 and 200 mg with 3 (23), 10 (32) and 6 (22) pts (cycles) per DL. MTD: 200 mg with GI DLT (vomiting, nausea, anorexia) in 2/6 pts (33%) and 2/22 cycles (9%). No DLT was observed at 150 mg.
Tumor activity: No OR was observed. Long-lasting SD was seen in 3 pts treated with schedule 1 (RCC: 100 mg, 12 cycles; Pancreatic neuro-endocrine Ca: 100 mg, 10 cycles; Thyroid Ca: 200 mg, 11 cycles) and 2 pts treated with schedule 2 (Pancreas Ca: 100 mg, 9 cycles; RCC: 100 mg, 8 cycles).
PK: With a mean of 17.75 h (range 13–23) T1/2 is much longer than that of other fluoropyrimidines. The PK profile of Cmax and AUC is linear over the studied DLs. There is no measurable accumulation of FT in the plasma over multiple cycles of schedule 2.
Conclusions: The principal DLT of FT is similar to that of other fluoropyrimidines. Dosing schedule 2 with a recommended dose of 150 mg orally once-a-day was chosen for Ph II "proof-of-concept" studies in selected solid malignancies.