Publication
Translational research

1480 PUBLICATION

Curcumin sensitizes human colon cancer cells to EGF-receptor related protein (ERRP)-mediated apoptosis

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Colorectal neoplasia is the second most common cancer in the United States with about 140,000 newly diagnosed cases per year. Mortality still remains unacceptably high. Development of other therapeutic strategies is, therefore, warranted. Numerous dietary and pharmacological agents have been proposed as alternative strategies for treatment and prevention of colorectal cancer. Curcumin, an active ingredient of turmeric that inhibits growth of malignant neoplasms both in vitro and in vivo has a promising role in colon cancer prevention. ERRP (EGF-R Related Protein), which we recently isolated and characterized as a negative regulator of EGF-R, is a potential therapeutic agent for colorectal cancer (Gastroenterology 124: 1337–1347, 2003). The goal of this study is to determine whether curcumin will sensitize colon cancer cells to growth inhibition by ERRP. To test this postulation, we utilized HCT-116 human colon cancer cells. The cells were pretreated with curcumin (10 μM) for 24 h, subsequently incubated with ERRP (5 μg/ml) for another 24 h. Additional incubations were performed with curcumin or ERRP alone or in their absence (control). Cell proliferation was assessed by MTT while apoptosis levels were determined by ELISA-based DNA fragmentation assay as well as by immunocytochemical methods. In addition, the levels of tyrosine phosphorylated (activated) forms of EGFR and IGF-1R were determined. Curcumin and ERRP by themselves caused 74 and 80% inhibition of cell proliferation, respectively, over the untreated controls, whereas pretreatment with curcumin resulted in greater than 90% inhibition by ERRP. With respect to apoptosis, curcumin and ERRP caused 75 and 120% apoptosis, respectively, compared to the untreated control, while combination therapy resulted in 150% increase in apoptosis. Together, our data suggest that pretreatment with curcumin sensitizes colon cancer cells (HCT 116) to the growth inhibitory effect of ERRP. The higher inhibition of growth by the combination therapy could partly be attributed to downregulation of EGFR and IGF-IR signaling pathways. We observed that whereas curcumin treatment resulted in attenuation of activation of both EGFR and IGF-IR, ERRP inhibited activation of EGFR, but had no effect on IGF-IR. We conclude that curcumin sensitizes colon cancer cells to growth inhibition by ERRP, and is the consequence of attenuation of two distinct signaling pathways: EGFR and IGF-IR.

1481 PUBLICATION

Erucylphosphocholine increases sensitivity of glioblastoma cell lines to the cytotoxic effects of ionizing radiation

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Novel treatment concepts are needed to improve poor prognosis of patients suffering from glioblastoma multiforme (GBM). GBM-tumours are often characterized by high intrinsic resistance against DNA-damaging drugs and ionizing radiation. Since resistance to DNA-damage-induced apoptosis can contribute to treatment failure novel agents targeting aberrant apoptosis signalling pathways of tumour cells may be suited to improve treatment efficacy. We could recently demonstrate that the membrane targeted anticancer drug erucylphosphocholine (ErPC), the prototype of intravenously applicable alkylphosphocholines, potently induces apoptosis in highly resistant GBM cell lines.
The aim of the present study was to analyse putative sensitizing effects of ErPC on radiation-induced cell death and clonogenic cell kill in human GBM cell lines in vitro.
Induction of apoptosis was evaluated in U87MG, A172 and T98G cells 24–72h after irradiation (2.5–10 Gy) with 6 MV photons from a linear accelerator and subsequent ErPC-treatment (T98G/A172 cells: 0–50 μM; U87MG cells: 0–100 μM). Cell death was quantified 24–72h after treatment by fluorescence microscopy using combined staining with Hoechst 33342 and propidium iodide. In addition, we also analyzed clonogenic cell survival upon combined treatment as a clinical relevant endpoint by standard clony formation assays. The biomathematical evaluation of putative additive or synergistic effects was performed by isobologram analysis.
While all GBM cell lines showed high intrinsic resistance against radiation-induced apoptosis, treatment with ErPC strongly increased radiation-induced cell death. T98G cells were most responsive to the combined treatment revealing highly synergistic effects and up to 90% cell kill. A172 cells showed additive to synergistic effects and U87MG cells maximum additive effects depending on the radiation and ErPC doses used. Importantly, in long term colony formation assays combined treatment of T98G cells resulted in a clinical relevant decline of clonogenic cell survival of about 4 orders of magnitude compared to radiation alone.
In conclusion, ErPC strongly increases sensitivity of GBM cell lines to the cytotoxic effects of ionizing radiation in vitro. For a proof of concept, in vivo experiments in a xenograft model will be performed in the future.
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1482 PUBLICATION

ELACYT™ (CP-4055), a novel cytotoxic agent, shows favourable safety and efficacy clinical results in the first phase study

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Background: ELACYT™ (CP-4055, Ara-C 5'-elaidic acid ester) is a novel cytotoxic agent which has shown wide preclinical antitumour activity in solid tumours. ELACYT™ is based on Lipid Vector Technology and has a different cellular uptake compared to Ara-C.
Methods: Patients (pts) with NSCLC, malignant melanoma or ovarian cancer received CP-4055 IV over 30 min on day (D)1–5, first in a 3 week (q3w), later in a q4w schedule. 3 pts were to be treated at each dose level (DL) in the absence of dose limiting toxicity (DLT). CP-4055, Ara-C and Ara-U were quantified in plasma and PK parameters were calculated on D1 & 4 of cycle 1. Safety assessments were performed by standard haematological and biological definitions. Antitumour response was assessed every 2 cycles.
Results: 30 heavily pretreated pts received 73 cycles of CP-4055 (median 2.4, range 1–8). The dose escalation was from 30 to 200 mg/m^2 by q3w, and after an amendment, 240 mg/m^2 by q4w. Six pts were treated at DL 175 mg/m^2 and above.
Safety: No unexpected AEs occurred, the most common being nausea, vomiting, fatigue, and anorexia, the majority of mild intensity. Neutropenia was the main haematological toxicity with nadir range D20–26. Two DLTs were reported in the q3w schedule, fatigue grade 3 and neutropenia grade 4 (175 and 200 mg/m^2). The MTD was 200 mg/m^2 when given by q3w. The study was amended due to the late nadir of neutropenia, and 6 pts were given 240 mg/m^2. All 6 pts experienced the same late onset of grade 4 neutropenia. The MTD was 240 mg/m^2 when given by q4w.
Efficacy: One melanoma pt (240 mg/m^2) was reported with partial response (31%), confirmed after 22 w. Stable Disease (SD) was reported in 11 pts, lasting 1.5–13 months, in all tumour types and at all DLs except 150 mg/m^2. One NSCLC pt was reported with SD lasting for 13 months. This pt had complete resolution of a pleural effusion. *PK*: The plasma exposure to Ara-C was generally low. The interpatient PK variability was low, and did not seem to change from the D1 to the D4 dose.
Conclusions: The MTD is 200 mg/m^2 when given D1–5 by q3w. This schedule is not recommended due to the late neutropenia. The MTD is 240 mg/m^2 and the recommended dose 200 mg/m^2 when given D1–5 by q4w. CP-4055 has a favourable safety profile, low interpatient PK variability and encouraging efficacy data. These results support the ongoing development of CP-4055, both in combination studies and as single agent therapy. A phase II study in malignant melanoma is initiated.

1483 PUBLICATION

ELACYT™ (CP-4055), a novel cytotoxic agent, administered according to three intermittent weekly or biweekly schedules to patients with advanced or metastatic solid tumours: phase I preliminary results

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Background: ELACYT™ (CP-4055, Ara-C-5'-elaidic acid ester) is a novel cytotoxic agent which has shown wide spectrum of preclinical antitumour activity in solid tumours. ELACYT™ is based on the Lipid Vector Technology and has a different cellular uptake compared to Ara-C. An initial phase I trial of a daily x 5 q3 weeks (w) schedule (sch) determined a recommended

dose of 175 mg/m^2/day and showed clinical activity. We report a multiple sch, parallel, dose intensity-guided phase I trial.
Methodology: Patients (pts) with solid tumours refractory to standard therapy received CP-4055 over 2 hours through a central venous catheter according to three sch: Days (D) 1, 8 q3w (sch A); D 1, 15 q4w (sch B); D 1, 8, 15 q4w (sch C). Dose escalation with dose levels (DL) defined according to dose intensity (DI) was used, DL1: 80 mg/m^2/w, DL2: 160 mg/m^2/w, DL3: 240 mg/m^2/w, DL4: 320 mg/m^2/w, DL5: 400 mg/m^2/w, DL6: 440 mg/m^2/w. Standard definitions of dose limiting toxicity (DLT) were used, including treatment delay >2w in the first 2 cycles. Inclusion is ongoing.
Results: 37 pts are treated to date at 6 DLs in 4 European centres between June 2004 and May 2005. Seven pts are still on treatment while 28 have discontinued due to progressive disease (PD) and 2 due to refusal. *Pt characteristics*: male/female: 20/17, median age 56 (range 35–72); WHO PS; 0: 12 pts, 1: 23 pts, 2: 2 pts. Principal tumour types: soft tissue and bone sarcoma, breast, colorectal, head and neck and GI; median of 3 lines of prior chemotherapy (range 1–5). *MTD*: No DLT has been observed. *Safety* (NCI-CTCAE ver 3): 32 pts assessed. The main toxicity, reported in all sch, is grade 1–2 anaemia (75% of pts; of these 25% had anaemia at baseline). Grade 1–2 nausea-vomiting is observed in 59% of all pts. Short lasting grade 3 neutropenia is observed in 2/17 pts at DL4 and DL5. No dose reduction required due to toxicity. *Efficacy*: Four sustained disease stabilizations (>3 months); breast cancer, prostate cancer, rectum cancer (sch A) and lung carcinoid (sch B).
Conclusion: Intermittent weekly or biweekly dosing of CP-4055 is well tolerated up to DI equal to 400 mg/m^2/w in the 3 sch tested. Patient accrual is ongoing at DL6 (440 mg/m^2/w)

1484 PUBLICATION

A metronomic treatment with oral vinorelbine in poor performance status patients with pretreated solid tumors

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Background: The safety profile and activity of oral vinorelbine (O-VNR) are comparable to that observed with i.v. formulation. It is known that the continuous infusion of drugs has possible advantages: administration of higher total dose; better tolerability; efficacy for a wider range of cell populations. An oral formulation can mimic the continuous infusion since the metronomic schedule implies the administration of low and repeated doses of drug.
The aim of this pilot study is to evaluate the faesibility of a metronomic schedule of O-VNR in poor performance status (PS) patients (pts) with pretreated solid tumors.
Patients and methods: From May 2004 till May 2005, 25 pts received O-VNR 30 mg fixed dose every other day for 12 weeks. Pts with tumor response or stable disease received another 12-week treatment until disease progression or toxicity or patient's refusal.
Pts' characteristics: median age 70 y (range 46–86); gender M/F 13/12; ECOG PS 2–3; tumor types: non small cell lung cancer 12, breast 3, unknown primary 2, sarcoma 3, prostate 1, ovary 2, kidney 1; rectum 1; metastatic sites were mainly viscera, soft tissue, brain; median number of previous lines 2.4 (range: 1–7).
Results: No WHO grade (G) 3–4 haematological or non-haematological toxicities have been recorded during 250 weekly treatments; vomit G1 has been reported in 3 pts.
Even if the activity was not a primary objective of the study, among the 11 pts who completed 12 weeks of O-VNR, 2 pts achieved partial response (breast, unknown primary tumor), 3 stable disease (lung, ovary) and 6 progression (kidney, sarcoma, lung and rectum).
Conclusions: In our preliminary experience the metronomic schedule of O-VNR is manageable: no significative toxicities have been recorded and no anti-emetic prophylaxis needed. This schedule might be used in phase II studies for malignancies, such as pretreated lung and breast carcinoma, where O-VNR showed activity and in poor PS pts even in early stages.

1485 PUBLICATION

The effect of rifampin on the pharmacokinetics of sunitinib malate (SU11248) in Caucasian and Japanese populations

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Background: Sunitinib malate is an oral multitargeted tyrosine kinase inhibitor which specifically inhibits VEGFR, PDGFR, KIT, RET and FLT3 and has demonstrated single agent antiangiogenic and antitumour activity in phase I and phase II clinical trials in a variety of advanced solid tumours, including gastrointestinal stromal tumour, metastatic renal cell carcinoma and metastatic breast cancer. Sunitinib is metabolised by cytochrome P450 (CYP) 3A4 to SU12662, an equipotent metabolite that is further metabolised by CYP34A. Thus, co-administration of sunitinib and drugs that induce CYP3A4 could lead to a reduction in sunitinib exposure.
Materials and methods: The pharmacokinetics (PK) of sunitinib were assessed in the presence and absence of rifampin (a potent CYP3A4 inducer) in an open-label, 2-way cross-over study in healthy male volunteers. Caucasian (n = 13) and Japanese (n = 15) volunteers were enrolled to enable a preliminary assessment of any effects of race. Volunteers were randomized to initial treatment with either Treatment A (a single oral dose of sunitinib 50 mg) or Treatment B (17 daily oral doses of rifampin 600 mg/day combined with a single dose of sunitinib 50 mg on Day 8); the alternative treatment regimen was administered following a 2-week washout period. Concentrations of sunitinib and SU12662 in plasma were determined using validated methods.
Results: Compared with administration of sunitinib alone, co-administration of sunitinib and rifampin resulted in a 4.8- and 4.7-fold reduction in sunitinib AUC_{last} and $AUC_{0-\infty}$, respectively, and a 2.3-fold reduction in sunitinib C_{max}. In terms of the active metabolite, a 1.3-fold increase in SU12662 AUC and a 2.4-fold increase in SU12662 C_{max} were observed following co-administration of sunitinib and rifampin. The PK results with and without co-administration of rifampin were similar in both ethnic groups.
Conclusions: The reduction in sunitinib exposure when the drug was co-administered with rifampin indicates that concomitant treatment of sunitinib with drugs that induce CYP3A4 should be avoided. The lack of racial differences supports the use of a common sunitinib starting dose and schedule in Caucasian and Japanese populations.

1486 PUBLICATION

A phase I combination study of split-dose short infusion trabectedin and doxorubicin administered every 21 days in patients with solid tumors

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Background: Trabectedin (YONDELIS™) is a minor groove binder with single agent activity in soft tissue sarcomas and ovarian carcinoma (OC) at doses of 1.3–1.5 mg/m^2 infused over 3 or 24 h every 3 weeks (Q3W). In combination with a standard dose of doxorubicin (DOX) trabectedin Q3W in 3 h could not be administered at dose higher than 0.8 mg/m^2 because of dose limiting neutropenia. This trial explores if a more frequent dosing allows to increase the trabectedin dose per cycle in combination with DOX.
Methods: 35 patients (pts) with advanced solid tumors were accrued into a multicenter phase I study delivering trabectedin in escalating doses on days 1& 8 and DOX (50 mg/m^2) on day 1Q3W. Dexamethasone (IV & OS) was given during and after trabectedin. Selection criteria were: $\leqslant$1 prior chemotherapy; maximum prior DOX 300 mg/m^2 and adequate renal, liver and hematologic function.
Results: 34 pts were treated with 103 cycles of the combination at trabectedin doses ranging from 0.3 to 0.8 mg/m^2. Median age was 56 yrs (range 32–71), PS was 0 in 74%, tumor type was (registered pts): OC, N = 26, sarcomas, N = 7, endometrial ca, N = 2. At the maximum trabectedin administered dose of 0.8 mg/m^2, 2 of 3 pts had acute hematologic dose limiting toxicity (DLT). The MTD was defined at 0.7 mg/m^{2}with 3 of 8 pts having DLT (2 hematologic, 1 liver). At 0.6 mg/m^2, 6 pts were entered in the dose escalation phase and 1 liver DLT observed (ALT > 2.5 x UNL on day 8). At trabectedin $\geqslant$0.5 mg/m^2 48% of cycles required dose reduction, delay or both, mainly because of neutropenia or delayed hematologic recovery. The combination was otherwise well tolerated: 1 pt had G4 reversible ALT ?, G3 nausea/vomiting, asthenia and reversible transaminase ? occurred in 9, 12 and 15% of pts, respectively. Of 25 treated OC pts relapsing after platinum-taxane therapy, 3 responded (12% 3–31 95% CL).
Conclusions: A higher total dose/cycle can be delivered with DOX Q3W when trabectedin is given on days 1&8 instead of 3-weekly, but this increase cannot be maintained beyond cycle 1 because of hematologic toxicity. Substituting DOX with a less neutropenic anthracycline might decrease the myelosuppressive interaction of the combination. A phase I trial with liposomal DOX is currently ongoing.