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