1487 PUBLICATION

Phase II evaluation of oxaliplatin (LOHP) and Lyposomal doxorubicin (PLD) as salvage chemotherapy in advanced solid tumors

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Objectives: In a previous phase I study (Anticancer drugs 2003; 14: 633–8) we found the maximally tolerated dose of Oxaliplatin (LOHP) combined with fixed doses of stealth pegylated liposomal doxorubicin (PLD). With this combination, we observed an overall response rate of 55% in heavily treated patients with recurrent ovarian cancer. As both drugs have been shown to be very active in a variety of solid tumors, we performed an extended phase II study, using this drug combination in advanced tumors pre-treated with chemotherapy.
Patients and methods: Utilizing a Simon's optimal two-stage design, 54 patients with metastatic tumors (ovarian 56%, breast 24%, miscellaneous 20%), recurrent after treatment with 1 (13%), 2 (33%), 3 or more (54%) lines of chemotherapy were entered into the trial. Patients had a median age of 61 years (range, 41/81). Metastatic sites: peritoneum 38%, liver 24%, bone 20%, lung 10%, nodes 8%. Treatment was as follows: PLD 40 mg/m^2 over 40 minutes, LOHP 120 mg/m^2 over 2 hours. Both drugs were administered day 1, every 3 weeks. In order to prevent peripheral neuropathy and palmar-plantar erythrodysesthesia (PPE) the administration of drugs was preceded by the infusion of reduced glutathione, potassium and magnesium and, concomitantly with the infusion of PLD, upper and lower extremities were refrigerated.
Results: The 54 patients received 355 courses of chemotherapy (mean 6.5). Toxicity (WHO) in % of patients. Anemia G 1–2 (42%), neutropenia G 1–2 (45%), G 3 (15%), G4 (4%), (PPE) G 1–2 (48%), G 3 (7%). Amongst 54 evaluable patients, we observed an overall response rate (RR) of 59.2% (95% confidence interval, 45% to 72%). With a median follow-up of 17 months (range, 4–46) median time to progression (TTP) was 9.6 months (m), while median overall survival (OS) was 18 months. RR, TTP and OS for disease sites were as follows: ovary: RR 58%, TTP 9.6 m, OS 22.2 m; breast – RR 58%, TTP 8.1 m, OS not reached; miscellaneous: RR 63%, TTP 7.2 m, OS 9.8m.
Conclusions: A combination of PLD and LOHP administered with the above described procedure, has a manageable toxicity profile and can be safely given as outpatient chemotherapy for heavily pre-treated patients with relapsed tumors. A very promising anti-tumour activity was observed, not only in ovarian cancer, but in all other tumor types.

1488 PUBLICATION

Photochemical internalization of chemotherapeutic agents to circumvent multidrug resistance

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Multidrug resistance is the major confounding factor in solid tumour therapy. A novel way of circumventing resistance, in order to deliver higher levels of chemotherapy and hence increase efficacy, is photochemical internalization (PCI) of chemotherapeutic agents. PCI involves the co-administration of a photosensitizing compound that upon light activation induces the release of organelle-bound chemotherapeutic agents into the cancer cell cytoplasm. In this work, hypericin was used as the photosensitizing agent.
We aimed to determine firstly the relative effect of hypericin-induced phototoxicity on resistant bladder and breast cancer cell lines; and secondly to examine whether PCI using hypericin was able to potentiate the cytotoxicity of the chemotherapeutic drug mitoxantrone (MTZ) on the same cancer cells.
Bladder and breast cancer cells (MGHU1 and MCF7) and their resistant counterparts (MGHU1R and MCF7R) were exposed to increasing doses of MTZ or hypericin alone at incubation times up to 24 h, with and without blue light exposure at c. 400 nm, to determine appropriate doses for further PCI combination experiments. Cell viability was assessed by the MTT assay. MTZ alone (2 μg/ml) resulted in 60–85% cell killing in parental sensitive cell lines, with resistant cells exhibiting 3.5–11 times less cytotoxicity. Using hypericin, in the absence of MTZ, the sensitive and resistant cells lines exhibited no differential cytotoxicity following light exposure.
For PCI, hypericin doses (0.1, 0.2 μM) and light exposures were chosen that induced no significant cytotoxicity. In combination, the co-administration of hypericin (plus light exposure) with MTZ significantly increased the killing effect on multidrug resistant cancer cells, compared to MTZ alone ($p < 0.05$ for MGHU1R; $p < 0.05$ for MCF7R).
Our results are consistent with an additive or synergistic effect of the combined strategies. However, further investigations are warranted for elucidating the underlying molecular mechanisms.

1489 PUBLICATION

Determination of optimal times of delivery for improved acute tolerability of 5-fluorouracil, oxaliplatin and carboplatin in colorectal or lung cancer patients

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Background: Preclinical data have indicated that best tolerability resulted from dosing 5-fluorouracil (5-FU) during the rest span for and oxaliplatin (l-OHP) or carboplatin (CBDCA) 12 h apart. Chronomodulated infusion (chrono) based on these principles were better tolerated than constant rate infusion in cancer patients (pts).
Methods: The relevance of peak time of chrono delivery for tolerability was investigated in pts with advanced or metastatic colorectal or lung cancer treated with 5-FU-leucovorin (LV) and l-OHP or CBDCA. The drugs were infused according to a sinusoidally-varying infusion rate over 11.5 h for 4 days every 2 weeks. The delivery peak time of 5-FU-LV (700/850 - 300 mg/m^2/d) and that of l-OHP (25 mg/m^2/d) or CBDCA (50 mg/m^2/d) occurred 12 h apart. In Trials 1 & 2, 8 chrono schedules with peak times of drug delivery staggered by 3 h were tested in 114 previously-treated colorectal cancer pts; the main endpoint was the incidence of grade 3–4 toxicity over the initial 2 courses. In Trial 3, this endpoint was investigated in lung cancer pts randomized to receive one of 3 delivery schedules of chrono 5-FU-LV-CBDCA with times of peak delivery 8 h apart. The data of Trial 1 served to compute the optimal peak times of delivery of 5-FU-LV and l-OHP and their respective 90% confidence limits, using the bootstrap method. The data of Trials 2 and 3 were examined as validating sets.
Results: In Trial 1, chrono 5-FU-LV with a peak at 01:00 or 04:00 and l-OHP with a peak at 13:00 or 16:00 produced grade 3–4 toxicity in 16.7% vs 80% of the pts with peak delivery 12 h apart. Diarrhea, the main toxic effect, had a similar schedule-dependent pattern. The optimal peak times of chronomodulated infusion [90% Confidence Limits] were 03:57 [23:30 to 09:36] for 5-FU-LV and 15:57 [11:30 to 21:36] for l-OHP. This optimal time was confirmed in Trial 2 for colorectal cancer pts and in Trial 3 for lung cancer pts, with grade 3–4 neutropenia as main toxicity (7% vs 24%, $p = 0.047$). Tolerability was twice as good and optimal time window appeared to be narrower in men vs women.
Conclusions: This first time-finding study has identified an optimal time and its 90% CL for three widely used anticancer agents in cancer patients supporting the predictive value of preclinical models of chronotolerance. This trial design method is undergoing further validation for other agents in the Chronotherapy Group of the European Organisation for Research and Treatment of Cancer.

1490 PUBLICATION

Induction of Fas-L and down-regulation of tubulin are responsible for the cytotoxicity of apicularen A in HM7 colon cancer cell

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Apicularen A, a novel highly cytotoxic metabolite from the *myxobacterial genus chondromyces*, have been shown previously to cause growth inhibition in several types of cancer cell lines, and apoptosis in RAW 264.7
To determine the mechanism of cytotoxicity of apicularen A in colon cancer cell, effects of apicularen A on the cell growth and apoptosis-related molecules were examined in HM7 cells. Upon treatment with apicularen A, a time and dose dependent inhibition of cell growth was observed and this inhibition could be partially rescued by caspase-3 and pan-caspase inhibitor. Flow cytometric analysis showed that apicularen A caused cells to accumulate in sub-G_0/G_1phase. Although apicularen A induced DNA fragmentation, release of cytocrome c, translocation of AIF to nucleus, change of Bcl-2 and Bcl-X_L were not detected. The activation of caspase-3 was associated with caspase-8 activity and not with caspase-9. Apicularen A-induced apoptosis through a membrane-mediated mechanism was supported by up-regulation of Fas-L, but not Fas (CD95/APO-1). Total and polymerized β-tubulin amount, and mRNA level of β-tubulin were decreased, but *in vitro* polymerization of tubulin were not effected by apicularen A. Concurrently, immunofluorescence microscopy indicated that apicularen A treatment disrupted microtubule architectures and decreased density of microtubule, and cells had almost eccentric

nucleus for 72 hr treatment. Moreover, apicularen A showed potent activity against xenograft tumors of the colon cancer HM7 cells.
From the above results, it is indicated that the mechanism of cytotoxicity of apicularen A in HM7 colon cancer cells are apoptosis through activation of death-receptor pathway and down-regulaion of tubulin synthesis.
This study was supported by a grant of The Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea(00-PJ2-PG1-CD02–007).

1491 PUBLICATION

The usefulness of continuous administration of hypoxic cytotoxin combined with mild temperature hyperthermia, with reference to the effects on quiescent cell populations in solid tumors

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Purpose: To evaluate the usefulness of continuous administration of hypoxic cytotoxins in terms of targeting acute hypoxia in solid tumors and the significance of combination with mild temperature hyperthermia (MTH) (40ºC, 60 min), we examined the cytotoxic effects of singly or continuously administered tirapazamine (TPZ) or newly-synthesized quinoxaline oxide TX-402 (3-amino-2-quinoxalinecarbonitrile 1,4-dioxide) in combination with or without MTH in vivo. Further, we also analyzed the effects on total (= proliferating (p) + quiescent (Q)) and Q cell populations in solid tumors with our method for selectively detecting the Q cell response.
Materials and Methods: C3H/He mice bearing SCC VII tumors received a continuous administration of 5-bromo-2'-deoxyuridine (BrdU) for 5 days to label all P cells. The tumor-bearing mice then received a single intraperitoneal injection or 24 h continuous subcutaneous infusion of hypoxic cytotoxin, TPZ or TX-402, with or without MTH. On the other hand, to detect the changes in the hypoxic fraction (HF) in the tumors by MTH, another group of mice with or without MTH received a series of test doses of gamma-rays while alive or after tumor clamping. After each treatment, the tumor cells were isolated and incubated with a cytokinesis blocker (= cytochalasin-B), and themicronucleus (MN) frequency in cells without BrdU labeling (= Q cells) was determined using immunofluorescence staining for BrdU. The MN frequency in total tumor cells was determined from the tumors that were not pretreated with BrdU.
Results: The sensitivity to TX-402 was slightly higher than that to TPZ in both total and Q tumor cells. Continuous administration elevated the sensitivity of both total and Q cells, especially total cells. MTH raised the sensitivity of Q cells more remarkably than that of total cells in both single and continuous administrations. It was thought to be probably because of the higher dose distribution of hypoxic cytotoxin in intermediately hypoxic areas derived mainly from chronic hypoxia through MTH.
Conclusion: From the viewpoint of tumor control as a whole including both total and Q tumor cells, the continuous administration of hypoxic cytotoxin combined with MTH may be useful for sensitizing tumor cells in vivo.

1492 PUBLICATION

Pemetrexed combined with gemcitabine and cisplatin: a phase I study in patients with locally advanced or metastatic solid tumors

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Background: Combining pemetrexed (P, Alimta®) with gemcitabine (G) and cisplatin (Cis) may achieve a synergistic action by combining different mechanisms of action, overlapping spectra of clinical efficacy, and non-overlapping toxicities. The purpose of this study is to determine the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of four different schedules of PGC.
Methods: In the q3w schedule, PGCis was administered on day (d) 1 and G on d8 in a 21d cycle. In addition, the following schedules were studied: A: GP d1 and GCis d15 q 28 days; B: GCis d1 and PCis d15 q 28 days; C: PGC is on d1 q14d. P was administered intravenously (IV) over 10 minutes (min), G IV over 30 min, and Cis IV over 2 hours. Standard pre- and postmedications were used.
Results: In the q3w schedule, 4 of 12 patients (pts) experienced DLTs, dose escalation was stopped in favor of alternative schedules. To date, 12 pts were enrolled into 3 dose levels of A, 22 pts enrolled into 7 dose levels of B, and 4 pts enrolled into 1 dose level of C. Tumor types included: head and neck (8), prostate (4), mesothelioma (4), NSCLC (4), sarcoma (4), stomach (4), kidney (2), esophagus (2) and others (6). 4 DLTs occurred in 4 pts on dose level 3 of schedule A, 2 DLTs in 2 pts on dose level 6 of schedule B and 4 DLTs in 2 pts on dose level 1 of C (MTDs: delay of therapy due to thrombocytopenia in schedule A and fatigue in schedule C). In dose level B, full clinical doses of G and Cis have been reached and further dose escalation of P is ongoing. With a total of 31/64/11 cycles administered so far in schedules A/B/C 4/2/1 pts experienced G3 anemia, 3/4/0 pts experienced G3/4 thrombocytopenia and 9/16/3 pts experienced G3/4 neutropenia. G3/4 non-hematological toxicities included G3 fatigue (6), G3 rash (2), G3 dysphagia (1), G3 syncope (1), G3 elevation of transaminases (1), G3 stomatitis (1) and G3 diarrhea (1). In the q3w schedule 1 partial response (PR) and 7 stable diseases (SD) were observed. For the schedules A, B, and C tumor response is so far evaluable in 7/15/3 pts. 2 pts of schedule A and 6 pts of schedule B achieved a PR, 3/4/2 pts achieved SD.
Conclusion: The PGCis combination is feasible and demonstrates clinical antitumor activity. Tolerability of the combination is influenced by administration sequence. Schedule B appears to offer the best tolerability when compared to the q 3 wk schedule or schedules A and B. Further clinical development of PGCis is promising.

1493 PUBLICATION

New anti-neoplastic agent MK615, extracted from Japanese apricot, Ume, inhibits growth of pancreatic and biliary cancer

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Purpose: MK615 is a newly developed anti-cancer agent, extracted from Ume, a japanese apricot. In the present study, inhibitory effect of MK615 to pancreatic and biliary cancer cell lines was investigated.
Methods: Four pancreatic cancer cell lines, MIA, PANC-1, PK-45H, PK-1 and 3 biliary cancer cell ines, HuCCT1, NOZ-W, and OZ were cultured with MK615 at the concentration of (600, 300, 150, 0 μg/ml). After 48 hours of incubation, live cells were counted by MTT assay. Data are presented by % inhibition at each concentration of MK615 to 0 μg/ml, and are shown by (600, 300, 150 μg/ml).
Results: MTT assay revealed that MK615 effectively inhibits the proliferation of all pancreatic and biliary cancer cell lines. For pancreatic cancer cell lines, % inhibitions of MIA, PANC-1, PK-45H, and PK-1 were (28, 6, 0), (68, 9, 3), (46, 9, 0), and (46, 9, 0), respectively. For biliary cancer cell lines, % inhibitions of HuCCT1, NOZ-W, and OZ were (83, 50, 26), (68, 25, 12), and (64, 0.0) respectively.
Conclusion: MK615 effectively inhibits the proliferation of pancreatic and biliary cancer cell lines, especially at the concentration of 600 μg/ml. MK615 should be promising as new anti-neoplastic agent.

1494 PUBLICATION

Pharmacokinetic of a novel cytotoxic agent, ELACYT™ (CP-4055) given according to four different schedules in two phase I studies

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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. We report pharmacokinetic (PK) results from two European phase I studies, exploring a daily x 5 schedule (sch) and weekly and biweekly sch.
Methodology: Using standard dose-finding design, patients (pts) with solid tumours received CP-4055 as a 30min infusion, daily x q3 week (w), over a 30–200 mg/m^2/infusion dose range in Study 1, and as a 2h infusion in Study 2 according to 3 sch: Days (D)1, 8 q3w (sch A); D1, 15 q4w (sch B); D1, 8, 15 q4w (sch C) over a dose range 100 to 800 mg/m^2/infusion. PK was assessed on D1 and D4 (Study 1 only) of cycle 1. Samples were taken at: 0:00, 0:15, 0:30, 0:35, 0:45, 1:00, 1:30, 2:00, 2:30, 4:30, 7:30, 10:30, 24:30h in Study 1; 0:00, 1:00, 2:00, 2:05, 2:15, 2:30, 3:00, 4:00, 6:00, 9:00, 24:00h in Study 2. CP-4055, Ara-C and Ara-U were qualified.
Results: As of May 2005, 61 pts (Study 1:24, Study 2: 37) with a variety of malignancies were evaluable for PK at D1 and 24 pts at D4. Accrual is ongoing in Study 2. Results from 56 pts are presented. *PK over all dose levels*: Interpatient variability of CP-4055, Ara-C and Ara-U was generally