Materials and Methods: Xenograft model Tumours were implanted subcutaneously to Balb/c nu/nu mice. After reaching to 100–200 mm^3 of tumour volume, nude mice were dosed i.p. with CKD-516 and/or conventional drug on a q.w. schedule. Tumour volume was determined by measuring the diameters of tumours using caliper and calculated using the formula $ab^2/2$, where a is the long length and b is the short length of the tumour. Statistical analysis was performed using the Student's *t*-test.
Toxicity studies CKD-516 and/or conventional drugs are administered to Balb/c mice. Blood samples were collected in EDTA containing tubes for WBC differential counting;, BUN, GPT, GOT analysis.
Results: Carboplatin, a second-generation platinum-containing anticancer drug, is currently being used against human cancers but high-dose carboplatin chemotherapy can cause nephrotoxicity in cancer patients.
Low dose CKD-516 inhibited tumour growth significantly in NSCLC xenograft (3 mg/kg, IR = 67.8%). Whereas carboplatin failed to show strong anticancer effect (100 mg/kg, IR = 46.7%) with observable toxicity.
CKD-516 in combination with carboplatin showed a synergistic effect without increasing toxicities (ie, hematologic toxicity, nephrotoxicity, hepatotoxicity).
Paclitaxel is a mitotic inhibitor used to treat patients with lung, breast, ovarian cancer.
CKD-516 inhibited tumour growth against NSCLC xenograft (2.5 mg/kg, IR = 48.7%; 5 mg/kg, IR = 70.4%) without decrease of body weight gain. Whereas paclitaxel inhibited the tumour growth insignificantly (10 mg/kg, IR = 25.2%; 20 mg/kg, IR = 45.5%) with observed toxicity.
CKD-516 in combination with paclitaxel showed synergistic effect without increment of toxicities. The inhibition rate of the combination reached 89.4%.
Conclusions: In summary, CKD-516 has shown synergistic effects in combination with other anticancer agents. Thus, CKD-516 warrants further development to treat NSCLC patients.

1088 POSTER

Liposome-Encapsulated Hemoglobin Enhances Radiotherapy and Chemotherapy to Suppress Tumour Growth and Metastasis in Mice

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Background: Liposome-encapsulated hemoglobin (LEH) has been developed using technologies for encapsulation of concentrated human hemoglobin with high encapsulation efficiency as well as surface modification to achieve stability in circulating blood and a long shelf life. An affinity of LEH to oxygen can be widely modified chemically. A high O_2-affinity LEH ($P_{50}O_2$ = 10 mmHg, h-LEH) showed the improvement in cerebral infarction and cardiac infarction of animal models. As in radiotherapy, anticancer drugs, such as doxorubicin (DXR) but not 5-FU, require O_2 to be cytotoxic. We hypothesize that targeted O_2 delivery to tumour hypoxia by h-LEH may certainly enhance cancer therapy. The study was performed to assess the potential of h-LEH to overcome tumour hypoxia and to improve the effect of radiotherapy and chemotherapy.
Material and Methods: 20 Gy was given to mouse squamous cell carcinoma, SCCVII, grown in the leg of C3H/HeN mice. H-LEH or empty liposome was infused intravenously 30, 60, 90 and 120 min before irradiation. Tumour size was monitored thereafter to evaluate the suppression on tumour growth. H-LEH or empty liposome was infused in the additional mice with SCCVII tumour, which was excised various timing later for immunohistochemical staining for h-LEH and HIF-1α. DXR and S-1 (a novel oral 5-FU derivative) were applied on the Lewis Lung Carcinoma (LLC) grown in the leg of C57BL/6N mice. Daily administration of DXR (0.5 mg/kg, intraperitoneally) or S-1 (8 mg/kg, orally) was started 48 h after inoculation of LLC tumour for 2 consecutive weeks. H-LEH (5 mL/kg) was infused 2 h after each chemotherapy every other day for 2 weeks. After 2W treatments, mice were sacrificed for quantitative and macroscopical examinations of the tumour growth and lung metastasis.
Results: H-LEH was most effective when 10 mL/kg was infused before irradiation as compared to empty liposome or 5 or 20 mL/kg of h-LEH. SCCVII tumour growth was most suppressed when interval between h-LEH infusion and radiation was shortest, 30 min. As the result, 10 mL/kg of h-LEH infusion 30 min prior to radiation prolonged 5-time tumour-growth time from 20.0 days (radiation and empty liposome) to 26.5 days; $P < 0.01$, synergy ratio 1.42. H-LEH was detected in the tumour 6 to 24 h after infusion, when HIF-1α expression was reduced only in the h-LEH-treated mice. Administration of h-LEH or DXR alone had no effect on LLC tumour growth in the leg and metastasis in the lung. Addition of h-LEH to DXR resulted in 30.5% reduction of tumour weight ($P < 0.05$) and 41% reduction of lung metastasis ($P < 0.01$). While S-1 had a marked effect on both tumour growth (35% tumour weight reduction) and 62% reduction of metastasis, addition of h-LEH had no synergistic effect on the anti-tumour effect of S-1.
Conclusions: These results suggested that h-LEH may have the potential of synergistic action not only with radiotherapy but also with chemotherapy. Decreased expression of HIF-1α in the h-LEH-treated tumour may suggest targeted tumour oxygenation as a potential mechanism.

1089 POSTER

Apoptosis Induction by Low Voltage Electric Pulses

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Background: Electric fields have been widely used in a variety of *in vitro* and *in vivo* biotechnical applications. Electroporation, in particular, has been investigated as a novel anticancer therapy, known as electrochemotherapy. However, high-intensity electric fields may also cause undesirable side effects. On the other hand, previous studies reported that a moderate electric field induced apoptosis. Therefore, research into the cellular effects of exposure to mild electric fields is useful for cancer research.
Materials and Methods: *Cell culture* The B16 (mouse malignant melanoma) cell line/SCC-9 (human tongue cancer) cell line.
Exposure to LVEPs (Low voltage electric pulses) Cells were re-suspended in RPMI-1640 at a concentration of $2-3\times10^6$ cells/ml. The cell suspensions (400 μl) were transferred to electroporation/fusion chambers. Relatively low-voltage (7.5 V/mm) square wave consecutive pulses were applied using a function generator.
Assay A flow cytometry apoptosis detection kit (Annexin V-FITC/7-AAD) was used to identify apoptotic and necrotic cells. Caspase-3, -8 and -9 activities were measured using an activity detection kit with FITC fluorescence by flow cytometry.
Results: *Induction of apoptosis and cell death*: On average, apoptosis occurred in 2.8% of the control cells, 3.2 −10.5% of cells exposed to electric pulses, and 7.4% of the cisplatin-treated cells.
Caspase-3, -8 and -9 activity: Caspase-3 activation increased within 3 h after electric pulse exposure and increased gradually until 24 h. Thereafter, caspase-3 activation decreased gradually, but did not recover to the control level at 48 h. Moreover, LVEPs activate both caspase-8 and -9 (*i.e.,* both the cell death receptor and mitochondrial pathways).
Conclusion: LVEPs induce apoptosis in a manner that is primarily dependent upon caspase-3 activation through caspase-8 and -9 activation. LVEP-induced apoptosis may result from membrane dysfunction that disrupts the transport of Ca^{2+} and extracellular substances, which are potent caspase activators. However, further studies are required to define the electric pulse conditions that most effectively induce apoptosis and to elucidate the detailed mechanism of LVEP-induced apoptosis.

1090 POSTER

The Polymorphism of the IGF-1 Gene in Patients With Breast Cancer and Effects on Prognostic Factors

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Background: The insulin-like growth factor I (IGF-I) is known to have a role in the risk of breast cancer because of stimulating cell proliferation, differentiation and apoptosis. However, the relation between IGF-I gene polymorphism and the clinicopathological variables of breast cancer remains unknown. We aimed to evaluate the association between CA repeat polymorphism of IGF-I gene in 76 breast cancer patients.
Methods: The IGF-I (CA) repeats studied with polymerase chain reaction by using proper primers belonging to these gene areas from DNA samples.
Results: Of the 76 patients, 40 (52.6%) had non 19-non 19 homozygote, 12 (15.8%) had non 19−19 heterozygote, and 24 (31.6%) had 19−19 homozygote. There was no relationship between age, body mass index, menopausal status, stage, bilaterality, estrogen receptor status, c-erb B2 overexpression, histological grade, tumour size and the CA repeat polymorphism of IGF-I gene. The non 19- non 19 homozygote were more common in patients without lymph node involvement (70.8%, $p = 0.07$) and in patients without progression (63.2%, $p = 0.044$). In survival analysis, the carriers of non 19- non 19 homozygote had longer PFS (mean 114.3+19 months, $p = 0.13$) and OS (142.9+22.7 months, $p = 0.05$) than the other groups.
Conclusions: These results suggest that the non 19-non 19 carriers have some favorable effects on disease progression and survival.