M. LOHP and PTX induced G_2/M arrest, 5-FU S phase increase, and ZD1839 G_1 increase in a concentration-dependent manner.A previously developed cytostatic TPi model (Jpn J Cancer Res 91:1303) was used to assess the contribution of cell cycle arrest to overall growth inhibition, and 64% and 80% of the overall growth inhibition at IC_{80} after 72hr was attributed to cell cycle arrest for LOHP and PTX, respectively. When combined, PTX+ZD1839 showed the greatest synergism and LOHP+ZD1839 was also synergistic. The cell cycle effect and apoptosis induced by PTX were potentiated by the coadministration of ZD1839. This study demonstrates the antitumor activity of ZD1839 against human gastric carcinoma cells and its synergistic interaction with LOHP and PTX. These results provide a preclinical rationale for future clinical development of ZD1839 and its use in combination with LOHP or PTX against MMR deficient human gastric cancers that express EGFR.

[OA-3] [10/18/2002 (Fri) 11:50 - 12:00 / Hall A]

Pharmacodynamics of anticancer activity of tirapazamine and paclitaxel against human NSCLC

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Hypoxia in solid tumors is known to contribute to intrinsic chemoresistance. Tirapazamine(TPZ), a hypoxia-selective cytotoxin, showed synergism with radiation or cytotoxic agents. Paclitaxel(PTX) is a highly active anti-cancer agent against Non small cell lung cancer(NSCLC), however, due to poor penetration into central hypoxic region of tumor tissue, combination with TPZ has been suggested to enhance its efficacy. We investigated pharmacodynamics of cytotoxicity, cell cycle arrest and apoptosis induced by TPZ and PTX in monolayers and histocultures of A549 human NSCLC cells. Hypoxic cytotoxicity ratios (HCR) of TPZ in monolayers increased with longer drug exposure. In monolayers, the values of n50(CnxT=k model, at 50% inhibition level) were not greater than 0.5 for TPZ and PTX, indicating greater importance of exposure time than drug conc. In monolayers, TPZ and PTX induced conc-dependent cell cycle arrest(G2/M), and hypoxic condition (2% O2) potentiated cell cycle effect of TPZ by 10 folds compared to normoxic condition. In histocultures, n50 for TPZ was 1.3, indicating greater importance of drug conc than exposure time. Cytotoxicity and cell cycle effect of PTX were significantly reduced in histocultures. However, cell cycle effect induced by TPZ in histocultures was similar to that in monolayers under hypoxia. PTX and TPZ induced apoptosis in cells in G1/S phase and G2/M phase, respectively. These data indicate that (1) pharmacodynamics of TPZ and PTX in monolayers is significantly different from that in 3-dimensional histocultures, which represents in vivo solid tumors, and (2) both TPZ and PTX induced G2/M arrest, but different cell cycle-specific apoptosis was observed. Grant 2000-0-214-001-3 from KSEF.

[OA-4] [10/18/2002 (Fri) 12:00 - 12:10 / Hall A]

The Differential Roles of Glutamine Synthetase in Methylmercury Neurotoxicity

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Methylmercury (MeHg), a potent neurotoxicant, produces neuronal death that may be partially mediated by glutamate. Glutamine synthetase (GS), a glial-specific enzyme, catalyzes the synthesis of glutamine from glutamate and ammonia and is associated with ischemic injury and neurological diseases. Objectives of this experiment are to investigate whether *in vivo* and *in vitro* MeHg exposure have adverse effects on GS and whether duration of exposure to MeHg and glutamate co-treatment play a role in MeHg-induced toxicity. GS activity was measured in cell-free brain homogenate of untreated rats, mice treated with MeHg (2, 4, 10 mg/kg for 1 days), primary cultured glial cells,

purified astrocytes, and rats treated with MeHg (2, 4, 10 mg/kg for 3 days). MeHg exposure (0.1 to 100 μM) to cell-free brain homogenate produced dose-dependent decreases of GS activity. In mice given MeHg (2, 4, 10 mg/kg for 1 days), GS activity was inhibited in 4 or 10 mg/kg MeHg-treated groups. In cultured mixed glial cells, however, MeHg exposure (0-10 μM, for 6 days) resulted in dose-dependent increases of GS activity. In the mixed glial cells exposed to 5 μM MeHg only for 6 days, GS activity was significantly increased (2-fold), with no effects observed in MeHg-exposed cells for 6 to 48 hr. In primary cultures of mixed glial cells and astrocytes treated with MeHg (0, 5, and 10 μM), dose-dependent increases of GS activity were reconfirmed. GS activity was also significantly increased in frontal cortex and caudate nucleus of 4 or 10 mg/kg MeHg-treated rats for 3 consecutive days. To investigate the effect of glutamate on MeHg-induced GS activity, MeHg (10 μM) and glutamate (0.5 or 100 μM) were co-treated to the mixed glial cells and astrocytes for 6 days. Exposure of glutamate (0.5 or 100 μM) to mixed glial cells or astrocytes has no effect on GS activity. These data showed the differential effect of MeHg on GS activity in cell-free brain homogenate, cultured mixed glial cells, astrocytes, and MeHg-treated rodents, indicating that increases of GS activity are related to repeated (long-term) exposure to MeHg.

[OB-1] [10/18/2002 (Fri) 12:10 - 12:20 / Hall A]

Role of Kupffer Cells in Hepatic Drug Metabolizing Dysfunction during Polymicrobial Sepsis

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Although hepatocellular dysfunction occurs during sepsis, the mechanism responsible for this remains unclear. Since Kupffer cells provide signals that regulate hepatic response in endotoxin and inflammation, the aim of this study was to investigate the role of Kupffer cells in the alterations in the hepatic microsomal drug metabolizing function during sepsis. Rats were subjected to polymicrobial sepsis by cecal ligation and puncture (CLP) followed by fluid resuscitation. The gadolinium chloride ($GdCl_3$, 7.5 mg/kg), inactivator of Kupffer cells, was injected intravenously at 48 h and 24 h prior to surgery. Liver samples were taken 2 h and 6 h (early sepsis) and 24 h (late sepsis) after CLP for measurement of activities of cytochrome P-450 (CYP 450) isozymes and RT-PCR analysis of mRNA for CYP 450's genes. Activities of CYP 1A1, 1A2 and 2B1 in liver microsomal fraction were measured as 7-ethoxyresorufin O-deethylase, methoxyresorufin O-demethylase, and pentoxyresorufin O-dealkylase activities, respectively. Aniline p-hydroxylase activity (CYP 2E1) was determined by measuring the formation of p-aminophenol. Serum alanine aminotransferase activity in all experimental groups was unchanged. However, in CLP rats, serum aspartate aminotransferase activity and lipid peroxidation levels were significantly elevated after 24 h of CLP and the increase in lipid peroxidation was suppressed by $GdCl_3$ treatment. Total CYP 450 content was significantly decreased after 24 h of CLP but $GdCl_3$ had little effect on total CYP 450 content. NADPH-CYP 450 reductase activity reduced after 6 h of CLP and again after 24 h of CLP. $GdCl_3$ prevented the decrease in NADPH-CYP 450 reductase activity after 24 h of CLP. CYP 2B1 activity in all experimental groups was unchanged. CYP 1A1 and CYP 2E1 activities were both significantly decreased 24 h after CLP, which were prevented by $GdCl_3$ treatment. CYP 1A2 activity was decreased 2 h and 24 h after CLP. $GdCl_3$ restored CYP 1A2 activity to the level of sham-operated rats. mRNA level for tumor necrosis factor-α (TNF-α) in CLP rats was significantly increased throughout the experiment.$GdCl_3$ prevented the increase in TNF-α mRNA 24 h after CLP. In contrast, mRNA levels for NADPH-CYP 450 reductase, CYP 1A2 and CYP 2E1 were significantly decreased 24 h after CLP, which were prevented by $GdCl_3$. We conclude that both the decreased activity of CYP 450 isozymes and the down-regulation of CYP 450's genes occur during the late stage of sepsis. Kupffer cells may be responsible for producing hepatocellular dysfunction during sepsis.

[OC-1] [10/18/2002 (Fri) 16:00 - 16:10 / Hall B]