derivatives. Five sets of primers were designed and one set was selected for RT-PCR. We have found that QIAamp viral RNA isolation kit was the most efficient extraction kit for these systems when several PCR conditions such as annealing temperature, reverse transcription temperature and MgCl2 concentration, etc. were optimized. The sensitivity was calculated to be 100 IU/ml and HCV RNA negative plasma pools showed negative. Both in-house method and COBAS amplicor HCV 2.0 showed positive for window period samples. ELISA-confirmed positive samples also provided 80.6% positive rate. With a spiking of HCV to albumin, immunoglobulins and coagulation factors, the in-house method can detect up to 100IU/ml. Meanwhile, COBAS amplicor HCV 2.0 afforded a lower sensitivity in high concentrated intramuscular immunoglobulins to 500IU/ml. Results of our investigation confirm that the in-house NAT appears to be a highly sensitive and specific method, which is reliable for plasma as well as for plasma-derived medicinal products.

[PB2-6] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Alteration of Hepatic Drug Metabolizing Function after Traumatic Injury

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The aim of present study was to investigate effects of blunt trauma on alterations in cytochrome P-450 (CYP)-dependent drug metabolizing function and to determine the role of Kupffer cells in the hepatocellular dysfunction Rats underwent closed femur fracture (FFx) with associated soft-tissue injury under anesthesia. Control animals received only anesthesia. To deplete Kupffer cells in vivo, gadolinium chloride (GdCl3) was injected intravenously via the tail vein at 7.5 mg/kg body wt. 1 and 2 days before surgery. At 72 h after FFx, serum alanine aminotransferase (ALT) activity was increased, and this increase was attenuated by GdCl3 pretreatment. Serum aspartate aminotransferase (AST) and lipid peroxidation levels were not changed by FFx trauma. Hepatic microsomal CYP content and aniline p-hydroxylase (CYP 2E1) activity were significantly decreased, which were not prevented by GdCl3. The level of CYP 2B1 activity was decreased by Kupffer cell inactivation, but not by FFx. There were no significant differences in the activities of CYP 1A1, CYP 1A2 and NADPH-CYP reductase among all experimental groups. Our findings suggest that FFx trauma causes mild alteration of hepatic CYP-dependent drug metabolism and Kupffer cells are not essential for the initiation of such injury.

[PB2-7] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Effect of Trolox C on CYP450 Isozymes Activity and Expression in Hepatic Ischemia/Reperfusion

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The present study was done to determine the effect of trolox C, a hydrophilic analogue of vitamin E, on alteration in cytochrome P-450 (CYP)-dependent drug metabolism during ischemia and reperfusion. Rats were subjected to 60 min of hepatic ischemia and 5 h of reperfusion. Rats were treated intravenously with trolox C (2.5 mg/kg) or vehicle (PBS, pH 7.4), 5 min before reperfusion. Serum alanine aminotransferase and lipid peroxidation levels were markedly increased after ischemia and reperfusion. This increase was significantly suppressed by trolox C. Cytochrome P-450 content and NADPH-cytochrome P-450 reductase activity were decreased by ischemia/reperfusion, and restored by trolox C. Furthermore trolox C significantly increased NADPH-cytochrome P-450 reductase protein expression. There were no significant differences in ethoxyresorufin *O*-deethylase (CYP 1A1) and methoxyresorufin *O*-demethylase (CYP 1A2) activities among all experimental groups. While pentoxyresorufin *O*-dealkylase (CYP 2B1) activity was decreased, aniline *p*-hydroxylase (CYP 2E1) activity and its protein expression was increased by ischemia and reperfusion, which were prevented by trolox C. Our findings suggest that ischemia and reperfusion induces hepatic microsomal dysfunction by increasing lipid peroxidation, and trolox C ameliorates this change through its free radical scavenging activity.

[PB2-8] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Kupffer Cells Are Responsible for Producing Hepatic Microsomal Drug Metabolizing Dysfunction during Trauma and Sepsis

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Sepsis remains the leading cause of morbidity and mortality following trauma. Although hepatocellular dysfunction occurs during trauma and sepsis, the mechanism responsible for this remains unclear. We investigated the role of Kupffer cells in the alterations in microsomal drug metabolizing function during trauma and sepsis. Rats were subjected to trauma by femur fracture (FFx). After 72 h, polymicrobial sepsis was induced by cecal ligation and puncture (CLP). To inactivate Kupffer cells, the gadolinium chloride ($GdCl_3$, 7.5 mg/kg) was injected intravenously at 1 and 2 days prior to surgery. Liver samples were taken 2 h and 6 h (early sepsis) and 24 h (late sepsis). After CLP alone, serum AST activity and lipid peroxidation level were elevated 24 h after CLP and started to increase 2 h and remained constant upto 24 h after CLP in FFx + CLP, which were suppressed by $GdCl_3$. Total cytochrome P-450 (CYP 450) content was decreased in CLP alone. This decrease was potentiated after FFx + CLP. NADPH-CYP 450 reductase activity was reduced 6 h and again after 24 h of CLP in both CLP and FFx + CLP, which were prevented by $GdCl_3$ treatment. CYP 2B1 activity was decreased 2 h in FFx + CLP and $GdCl_3$ restored this decrease. CYP 1A1 activity was decresed 24 h in CLP alone and 6 h and 24 h after CLP in FFx + CLP. CYP 2E1 activity was decreased 24 h in CLP alone and remained depressed throughout the experiment in FFx + CLP, which were prevented by $GdCl_3$. CYP 1A2 activity was decreased 24 h in CLP alone and 6 h after CLP in FFx + CLP. We concluded that sepsis alone decreases the activity of CYP 450 isozymes during late stage of sepsis, while sequential injury potentiates this decrease during early and late sepsis. Activation of Kupffer cells may contribute to hepatocellular dysfunction.

Poster Presentations - Field B3. Neuroscience

[PB3-1] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Adenosine inhibits the death in immunostimulated murine astrocytes deprived of glucose

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Adenosine has been associated with protection of neurons from noxious stimuli both by receptor- and non receptor-mediated mechanisms. Previously we have reported that immunostimulated astrocytes were highly vulnerable to glucose deprivation. In the present study we investigated the effect of adenosine and related nucleotides on the susceptibility of immunostimulated astrocytes to glucose deprivation. While neither 12-h glucose deprivation nor 2-day treatment with IFN-γ and LPS altered the viability of astrocytes, significant death of IFN-γ/LPS-treated astrocytes was observed after 4-h glucose deprivation. The augmented astrocyte death was blocked by adenosine with an apparent EC50 value of 20 mM. However, adenosine receptor agonist R-PIA or CHA did not inhibit the augmented cell death. Moreover, adenosine receptor antagonists DPCPX, XAC or DMPX did not alter the augmented death, ruling out the involvement of adenosine receptor in this process. Other purine nucleotides including guanosine, inosine, AMP, ADP and ATP, but not pyrimidine nucleotides such as cytosine, showed similar protective effects. Intracellular ATP level rapidly decreased prior to the release of LDH in immunostimulated astrocytes deprived of glucose. Adenosine and other purine nucleotides inhibited the loss of intracellular ATP. Since high micromolar concentrations of ATP and adenosine nucleotides were released in cerebral hypoxic/ischemic regions, ATP, adenosine and their metabolites may protect the astrocyte death by restoring intracellular ATP level, at least in our experimental systems.

[PB3-2] [10/17/2002 (Thr) 13:30 - 16:30 / Hall C]

Neuroprotective effects of baicalein, baicalin, and wogonin in primary cultured rat cortical cells