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ADMINISTRATION OF BRANCHED CHAIN AMINO ACIDS TO HYPERAMMONEMIC CIRRHOTIC ANIMALS IS ASSOCIATED WITH WORSENING OF HYPERAMMONEMIA AND INCREASE IN BRAIN OEDEMA

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Introduction Despite branched chain amino acids (BCAA) being used for over 30 years, its efficacy in treating hyperammonemia remains controversial. Recent studies suggest that the use of BCAA in cirrhosis is associated with increased glutamine levels, with a paradoxical increase in arterial ammonia. The mechanism of this remains uncertain. The administration of phenylacetate during increased glutamine levels reduces hyperammonemia through excretion of the ammoniagenic glutamine as phenylacetylglutamine (PAGN). We hypothesised that administration of phenylacetate with BCAA would reduce ammonia concentrations by stimulating ammonia capture by glutamine synthetase (GS) with subsequently phenylacetate removing the increased glutamine as PAGN, excreted into urine.

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Method Sprague–Dawley (n=22) bile duct ligated (BDL) or sham operated rats were studied 4 weeks post surgery (262±11.51 g). Groups: Sham (n=6); BDL+saline (n=6); BDL+BCAA (0.3 g/kg/day; n=6); BDL+BCAA dose+phenylacetate (BCAA+P, 0.3+0.3 g/kg/day; n=4). Treatment was for 3 days prior to sacrifice. Arterial ammonia (COBAS), GS and Glutaminase (GA) enzyme activity was measured (using enzymatic methods) in the muscle, gut, and the liver and, the brain water was measured using the dry weight technique.

Results Plasma ammonia was significantly higher in the BCAA group compared with controls (BCAA=118±20.8; vs sham43±8.3 μM p<0.01) and remained elevated in the BCAA+P animals (78.5±31 μM) which was associated with significantly higher brain water (BCAA=79.96±0.3%; BCAA+P=79.78±1.1%; sham=75.9±0.1% p<0.02 & p<0.05 respectively). GS activity in muscle increased in all BDL groups (p<0.05 in each case), and appeared to further increase following BCAA administration (BDL=7.13±1.7 IU/mg; BCAA=9.63±2.4 IU/mg, p=0.1). Hepatic GS activity was significantly reduced in all BDL groups (p<0.05 for each vs sham), with no apparent effect of BCAA. Duodenal GA activity was elevated with BCAA treatment, and significantly higher in the BCAA+P group (BCAA+P=1.66±0.4 IU/mg vs sham 0.43±0.07 IU/mg, p<0.01).

Conclusion The administration of BCAA resulted in an unexpected worsening of hyperammonemia and brain water which was further exacerbated by addition of phenylacetate. The deleterious effect of BCAA may result from increased muscle glutamine generation, which can cycle to generate ammonia from intestinal GA. The lack of beneficial effect of additional phenylacetate may be due to further increased GA activity. The interplay between GS and GA is pivotal in regulating ammonia levels in cirrhosis, and effectiveness of new therapies aimed at ammonia must address how they alter the function of these enzymes.

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EVIDENCE OF DENDRITIC CELL DYSFUNCTION IN CIRRHOSIS AND ITS RESTORATION BY TOLL-LIKE RECEPTOR 4 ANTAGONISM

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Introduction Infection complicates the course of cirrhosis and is the main cause of admission of patients to hospital. Recent studies have shown evidence of immune paralysis during severe decompensation. Our studies have suggested that neutrophil dysfunction in alcoholic hepatitis patients may be associated with a defect of TLR4 signaling, which is a key pathogen response receptor.

Aim The aims of this study were to characterise cellular immune function in a bile-duct ligated (BDL) model of cirrhosis and evaluate the role of TLR4 by using a selective antagonist, STM 28. (STM28 was kindly gifted by Professor Ken-ichi Tanamoto, Division of Microbiology, National Institute of Health Sciences, Tokyo 158-8501, Japan).

Method 18 C57BL/6 mice were studied: Sham operated, BDL (2 weeks after ligation) and BDL treated with TLR4 antagonist STM-28 (20 ug IP, 5 days). Peripheral blood was stained with fluochrome-labelled antibodies specific for peripheral blood myeloid cells, dendritic cells (DC's, plasmacytoid and myeloid, expression of CD86 in myeloid); monocytes (residents and inflammatory); total granulocytes and neutrophils; CD4/CD8 T-cells and the expression of CD25 in CD4 T cell population; B cells and NK cells. Flow cytometric analysis was performed on a FACS Canto II. Biochemistry was measured spectrophotometrically.

Results A significant shift towards the myeloid subset of peripheral blood DCs was observed in BDL animals compared with sham (81% vs 54%, p=0.0002) accompanied by a significant decrease in the percentages of plasmacytoid DCs (2% vs 25%, p<0.0001), and an increased expression of CD86 in myeloid DC's (72% vs 55% p=0.05). There was an increase of TLR4 expression on monocytes and neutrophils (p=0.05). The percentage of CD3 T cell and CD8 T cell subpopulations were significantly lower only in BDL group (3% vs 14%, p=0.02; 1% vs 6%, p=0.02 respectively). No significant difference in NK cells was observed. TLR4 antagonist in BDL animals showed restoration towards plasmacytoid subset DCs, with a significant reduction in the percentage of myeloid DCs (67% vs 81%, p=0.003) but there was no significant difference in percentage of total myeloid cells, total granulocytes, neutrophils and monocyte populations. Liver function remained unaffected by the TLR4 antagonist.

Conclusion In cirrhosis, myeloid subtype DCs are expanded and there is relative depletion of plasmacytoid DC's and lymphopenia. Treatment with a TLR4 antagonist redresses a shift of dendritic cells from the myeloid back to plasmacytoid type. As the DC's play a pivotal role in antigen presentation, TLR4 antagonism may play an important therapeutic role in decreasing susceptibility of cirrhotic patients to infection.