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• BASIC RESEARCH •

Prostacyclin inhibition by indomethacin aggravates hepatic damage and encephalopathy in rats with thioacetamide-induced fulminant hepatic failure

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Abstract

AIM: Vasodilatation and increased capillary permeability have been proposed to be involved in the pathogenesis of acute and chronic form of hepatic encephalopathy. Prostacyclin (PGI_2) and nitric oxide (NO) are important contributors to hyperdynamic circulation in portal hypertensive states. Our previous study showed that chronic inhibition of NO had detrimental effects on the severity of encephalopathy in thioacetamide (TAA)-treated rats due to aggravation of liver damage. To date, there are no detailed data concerning the effects of PGI_2 inhibition on the severity of hepatic encephalopathy during fulminant hepatic failure.

METHODS: Male Sprague-Dawley rats weighing 300-350 g were used. Fulminant hepatic failure was induced by intraperitoneal injection of TAA (350 mg/(kg·d) for 3 d. Rats were divided into two groups to receive intraperitoneal injection of indomethacin (5 mg/(kg·d), n = 20) or normal saline (N/S, n = 20) for 5 d, starting 2 d before TAA administration. Severity of encephalopathy was assessed by the counts of motor activity measured with Opto-Varimex animal activity meter. Plasma tumor necrosis factor-α (TNF-α, an index of liver injury) and 6-keto-$PGF_{1\alpha}$ (a metabolite of PGI_2) levels were measured by enzyme-linked immunosorbent assay.

RESULTS: As compared with N/S-treated rats, the mortality rate was significantly higher in rats receiving indomethacin (20% *vs* 5%, P<0.01). Inhibition of PGI_2 created detrimental effects on total movement counts (indomethacin *vs* N/S: 438±102 *vs* 841±145 counts/30 min, P<0.05). Rats treated with indomethacin had significant higher plasma levels of TNF-α (indomethacin *vs* N/S: 22±5 *vs* 10±1 pg/mL, P<0.05) and lower plasma levels of 6-keto-$PGF_{1\alpha}$ (P<0.001), but not total bilirubin or creatinine (P>0.05), as compared with rats treated with N/S.

CONCLUSION: Chronic indomethacin administration has detrimental effects on the severity of encephalopathy in TAA-treated rats and this phenomenon may be attributed to the aggravation of liver injury. This study suggests that PGI_2 may provide a protective role in the development of fulminant hepatic failure.

Key words: Hepatic Encephalopathy; Fulminant hepatic failure; Prostacyclin; Indomethacin

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INTRODUCTION

Hepatic encephalopathy is a neuropsychiatric syndrome associated with acute liver failure, chronic parenchymal liver disease, or portal-systemic anastomosis[1-3]. The spectrum of symptoms may vary from subtle mental changes or a disrupted circadian rhythm to hepatic coma. The pathogenesis of hepatic encephalopathy has not been fully established and a delineation of pathogenetic factors for hepatic encephalopathy, as well as developing therapeutic modalities, are highly warranted today.

Hyperdynamic circulation observed in portal hypertension is characterized by pronounced vasodilatation, increased systemic and regional blood flows, and augmented cardiac index[4]. Vasodilatation and increased capillary permeability have been proposed to be involved in the pathogenesis of acute and chronic form of hepatic encephalopathy[5-8]. Recent studies demonstrated that excessive formation of nitric oxide (NO) and prostacyclin (PGI_2), the endogenous vasodilators synthesized by the vascular endothelium[9], are important contributors to the hyperdynamic circulation[10-13]. Our recent study[14] demonstrated that chronic inhibition of NO had detrimental effects on the severity of encephalopathy in thioacetamide (TAA)-treated rats due to aggravation of liver damage, suggesting a protective role for NO in the development of hepatic encephalopathy. Concerning the relationship between prostaglandins and the liver, some studies suggested that prostaglandins could protect the liver against the damage by a wide variety of toxins (carbon

tetrachloride, acetaminophen, galactosamine, alcohol), hypoxia, ischemia, and immune injury[15-23] although the mechanisms remain unclear. To date, there are no detailed data concerning the effects of PGI_2 inhibition on the severity of hepatic encephalopathy during the condition of fulminant hepatic failure.

The aim of this study was to investigate the effects of indomethacin, a PGI_2 inhibitor[24], on the severity of encephalopathy in rats with TAA-induced fulminant hepatic failure[25-27]. For more accurate quantification of the degree of hepatic encephalopathy, motor activities of rats were measured in automated open field boxes equipped with infrared cells[28-30]. Plasma levels of tumor necrosis factor-α (TNF-α, an index of liver injury), 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$ a metabolite of PGI_2), aminotransferase (ALT), total bilirubin, and creatinine were also determined.

MATERIALS AND METHODS

Animal model

Sprague-Dawley rats weighing 300-350 g were used. Fulminant hepatic failure was induced by intraperitoneal injection of TAA (350 mg/(kg.d) in normal saline, Sigma Chemical Co, St. Louis, MO, USA) for 3 consecutive days. Rats were divided into two groups to receive intraperitoneal injection of indomethacin (5 mg/(kg·d), $n = 20$)[24] or placebo (normal saline, N/S, $n = 20$) from two days prior to the starting of TAA administration and lasted for 5 d. To avoid hypoglycemia and electrolyte imbalance, subcutaneous injections of a solution containing 10% glucose water mixed with lactate ringer (25 mL/kg) was performed every 12 h after the first injection of TAA[31]. Motor activity measurements and blood sample collection were performed 3 d after the first administration of TAA. All rats were caged at 24 ℃ with a 12-h light-dark cycle and allowed free access to water and food. The experiments reported here were conducted according to the American Physiological Society guiding principles for the care and use of laboratory animals.

Measurement of motor activities

Motor activities in an open field were determined by using the Opto-Varimex animal activity meter (Columbus Instruments Inc., Columbus, OH, USA)[28-30]. The Opto-Varimex activity sensors utilize high-intensity, modulated infrared light beams to detect animal motion. Animals were housed in transparent cages (17 inches ×17 inches ×8 inches) through which 30 infrared beams pass in the horizontal plane, 15 on each axis. This device differentiates non-ambulatory movements (scratching, gnawing) from ambulation on the basis of consecutive interruption of the infrared monitoring beams. An additional row of infrared beams in the horizontal plane (15 on each axis) about 10 cm above the floor was used to count the vertical movements. During the activity measurements, animals had no access to food or chow. All studies were performed under strictly standardized conditions in the dark room for 30 min. The number of total movements, ambulatory movements, and vertical movements was separately recorded to reflect the motor activities of rats with fulminant hepatic failure.

Determination of plasma TNF-α, 6-keto-$PGF_{1\alpha}$, ALT, total bilirubin, and creatinine

After the abdominal skin was cleaned twice with iodine tincture and 70% alcohol, the abdomen was opened using a sterile technique. A 3 mL of blood sample was collected from the inferior vena cava into a pyrogen-free syringe containing 75 units of heparin sodium, then placed in an ice bath and transported immediately to the laboratory. Plasma was centrifuged at 4 ℃ and 2 935 *g* for 10 min, then stored at -70 ℃ in pyrogen-free polyethylene tubes for subsequent analysis within 6 wk.

We measured plasma TNF-α levels[32] with a commercially available solid phase sandwich enzyme-linked immunosorbent assay (rat TNF-α kits; R&D systems, Minneapolis, MN, USA) according to the protocol supplied by the manufacturer. The standards and samples were incubated with a TNF-α antibody coating a 96-well microtiter plate. The wells were washed with buffer and then incubated with anti-TNF-α antibody conjugated to horseradish peroxidase for 2 h. This was washed away and a yellow-brownish color was developed in the presence of tetramethyl benzidine chromogen substrate. The intensity of the color was measured in a Bio-kinetics reader (Bio-Tek Instruments Inc., Winooski, VT, USA) by reading the absorbance at 450 nm with a correction wavelength of 570 nm. We compared the samples against the standard curve to determine the amount of TNF-α present. All samples were run in duplicate. The lower limit of sensitivity for TNF-α by this assay was 5 pg/mL. The intra-assay and inter-assay coefficients of variation were 5.1% and 9.7%, respectively.

The measurement of 6-keto-$PGF_{1\alpha}$ was based on a competitive binding technique in which 6-keto-$PGF_{1\alpha}$ present in a sample competes with a fixed amount of alkaline phosphatase-labeled 6-keto-$PGF_{1\alpha}$ for sites on a sheep polyclonal antibody (6-keto-$PGF_{1\alpha}$ Kits; R&D systems, Minneapolis, MN, USA). During the incubation, the polyclonal antibody became bound to the donkey anti-sheep antibody coated onto the microplate. Following a wash to remove excess conjugates and unbound samples, a substrate solution was added to the wells to determine the bound enzyme activity. The color development was stopped and the absorbance was read at 405 mm in a Bio-kinetics reader (Bio-Tek Instruments Inc., Winooski, VT, USA). The intensity of the color was inversely proportional to the concentration of 6-keto-$PGF_{1\alpha}$ in the sample. All samples were run in duplicate. The lower limit of the sensitivity for 6-keto-$PGF_{1\alpha}$ by this assay was 1.4 pg/mL.

We determined plasma levels of ALT, total bilirubin and creatinine by colorimetric assay with a Clinical Systems 700 analyzer (Beckman Instruments, Fullerton, CA, USA).

Statistical analysis

Results were expressed as mean±SE. Statistical analyses for categorical variables were performed by Chi-square and Fisher exact tests. Student's *t*-test was used for continuous variables. Results were considered statistically significant at $P<0.05$.

RESULTS

Effects of chronic indomethacin administration on mortality rate in rats with fulminant hepatic failure

Four rats in the indomethacin group and one rat in the N/S group died during chronic administration of TAA before the measurement of motor activities. As compared with N/S-treated rats, the mortality rate was significantly higher in rats receiving chronic indomethacin administration (20% *vs* 5%, $P<0.01$).

Effects of chronic indomethacin administration on degree of hepatic encephalopathy in rats with fulminant hepatic failure (Figure 1)

In rats receiving indomethacin, the total movements were significantly decreased (indomethacin *vs* N/S: 438±102 *vs* 841±145 counts/30 min, $P<0.05$). The counts of ambulatory movements (256±62 *vs* 385±95 counts/30 min, $P = 0.29$) and vertical movements (28±12 *vs* 68±27 counts/30 min, $P = 0.20$) in the rats treated with indomethacin tended to be lower; however, the differences did not reach a significant level.

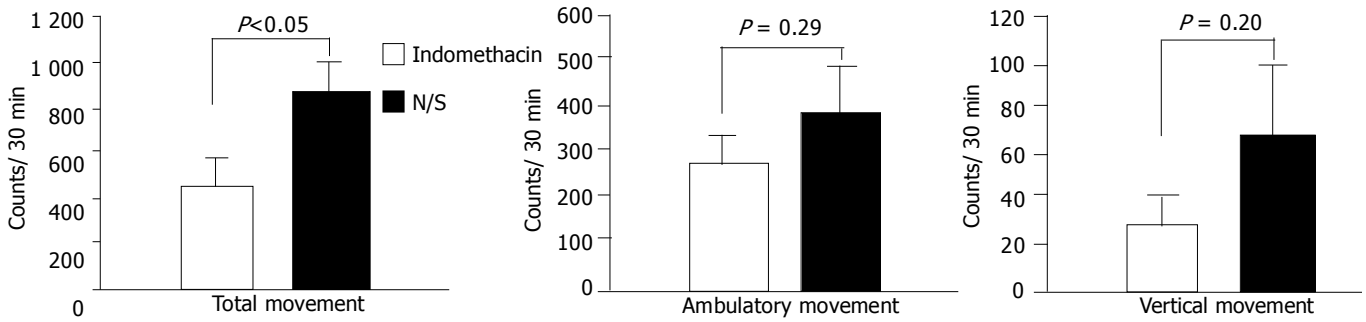

Figure 1 Comparison of the degree of hepatic encephalopathy by total, ambulatory and vertical movement counts between rats with fulminant hepatic failure treated with indomethacin (n = 19) or N/S (n = 16).

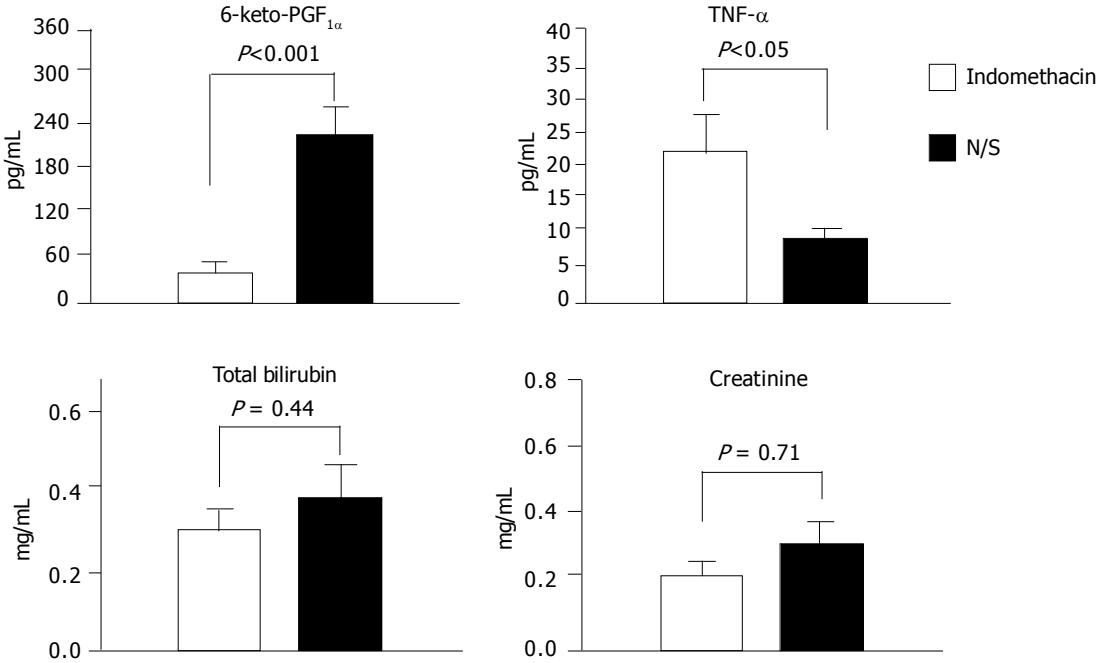

Figure 2 Comparison of the plasma levels of 6-keto-$PGF_{1\alpha}$, TNF-α, total bilirubin, and creatinine between rats with fulminant hepatic failure treated with indomethacin (n = 19) or N/S (n = 16).

Effects of chronic indomethacin administration on plasma levels of 6-keto-$PGF_{1\alpha}$, TNF-α, ALT, total bilirubin, and creatinine in rats with fulminant hepatic failure (Figure 2)

Rats treated with indomethacin had significantly lower plasma levels of 6-keto-$PGF_{1\alpha}$ (indomethacin *vs* N/S: 32±11 *vs* 213±47 pg/mL, P<0.001) and higher plasma levels of TNF-α (indomethacin *vs* N/S: 22±5 *vs* 10±1 pg/mL, P<0.05) as compared to N/S-treated rats. The indomethacin group of rats had a higher serum ALT (indomethacin *vs* N/S: 172±35 *vs* 141±31 IU/L, P>0.05); however, it did not reach a significant level. Serum levels of total bilirubin (indomethacin *vs* N/S: 0.3±0.1 *vs* 0.4±0.1 mg/dL, P>0.05) and creatinine (indomethacin *vs* N/S: 0.2±0.1 *vs* 0.3±0.1 mg/dL, P>0.05) were comparable between these two groups.

DISCUSSION

Regarding the relationships between the liver and arachidonic acid derivatives, in the last decade much has been learned between the interactions of liver and prostaglandins (PGs) under normal and pathological conditions. However, the puzzle is far from complete. Physiologically, the liver synthesizes all the major metabolites of the arachidonate cascade, including PGs. It has been found that PGs are involved in various physiological processes in the liver, including vasoregulation, platelet aggregation and mediation of inflammation[33]. As we stated previously, some studies suggested that PGs could protect the liver against damage by a wide variety of toxins (carbon tetrachloride, acetaminophen, galactosamine, alcohol), hypoxia, ischemia, and immune injury[15-23]. Although the precise mechanism underlying the cytoprotective effects of PGs in acute liver injury remains to be precisely defined, it may be related to the alleviation of vasoconstriction with tissue hypoxia, lipid peroxidation, and release of inflammatory cytokines or cytotoxic factors[34-36]. In agreement with previous studies[15-23], our current study showed that inhibition of PGI_2 by indomethacin during acute liver injury could aggravate hepatic damage. In addition, this is the first study which demonstrated that indomethacin administration could result in detrimental effects on encephalopathy in rats with TAA-induced fulminant hepatic failure. This finding was similar to NO inhibition in our previous research[14] suggesting that maintaining tissue oxygenation by vasodilators is important in fulminant hepatic failure. Besides, evidence from animal studies suggested indomethacin administration might affect neurotransmission and concentrations of neuropeptides in the brain[37-39]. Since our current study did not have data on the above issues, it is hard

to tell whether the deleterious effects of indomethacin injection on encephalopathy may be partially attributed to this and further studies are warranted.

Physiologically, TNF-α has been found to be a peptide mediator released by mononuclear cells on activation by endotoxin, tissue injury and tumor cells[40]. The metabolism of TNF-α was located within the liver after it was bound to a specific receptor[41]. Therefore, plasma levels of TNF-α, together with serum bilirubin, could be regarded as an index for evaluating the degree of hepatocellular damage and tissue necrosis.

Grading of hepatic encephalopathy is crucial in the research field for causally related pathogenetic factors. Various tests such as equilibrium test, corneal, auditory startle, and righting reflexes have been used but the interpretation of these tests may be subjective and cannot be quantified. Thus, a mechanical scoring system was applied in the present study. These tests are of great benefit in monitoring the minor changes in motivated behavior. On the basis of consecutive interruption of the infrared monitoring beams, we could differentiate non-ambulatory (scratching, gnawing) from ambulatory movements. The additional row of infrared cells above the plane could provide us more information concerning the movements toward the vertical direction. In the current study, the time interval from the first dose of TAA administration to the measurement of motor counts was fixed (72 h). The advantage of this model is that it can provide a therapeutic window for treatment modalities.

Indomethacin acts by inhibiting cyclooxygenase (COX), the rate-limiting enzyme in metabolic conversion of arachidonic acids into PGs. Two isoforms of COX have been identified[42] and both can be inhibited by indomethacin. COX-1 is expressed constitutively in most tissues and plays an important function in the protection of gastric mucosa and maintaining physiological homeostasis. COX-2 is thought to be the enzyme induced in inflammatory states and tumorigenesis. Recently, the intervention of selective COX-2 inhibitors was shown to preserve the anti-inflammatory efficacy of non-steroid antiinflammatory drugs (NSAIDs) and to achieve greater safety than traditional NSAIDs[43-45]. It is unknown whether using selective COX-2 inhibitors may have different effects on hepatic encephalopathy other than detrimental effect and this issue deserves further evaluations.

Results of preliminary clinical trials using PGs as therapeutic agents in patients with severe acute liver injury have been encouraging[46]. However, most of these trials were lack of control groups. To accept that PGs as a standardized treatment in some forms of liver injury, there is still much to learn about the intimate mechanisms responsible for the hepatoprotective effect. We acknowledge that this study may not be 100% extrapolated to patients with acute or chronic forms of hepatic encephalopathy in conditions of liver insufficiency. However, we want to point out that using NSAIDs in patients who potentially suffer from hepatic encephalopathy should be closely monitored.

In summary, chronic indomethacin administration had detrimental effects on the severity of encephalopathy in TAA-treated rats and this phenomenon may be partly attributed to the aggravation of liver injury. It is possible that prostacyclin inhibition may affect neurotransmission, brain microcirculation, or changes of blood-brain-barrier permeability. However, the detailed responsible mechanisms remained to be elucidated. Our current study suggests that PGI_2 may provide a protective role in the development of fulminant hepatic failure.

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REFERENCES

1 **Gammal SH**, Jones EA. Hepatic encephalopathy. *Med Clin North Am* 1989; **73**: 793-813
2 **Sherlock S**. Fulminant hepatic failure. *Adv Intern Med* 1993; **38**: 245-267
3 **Mousseau DD**, Butterworth RF. Current theories on the pathogenesis of hepatic encephalopathy. *Proc Soc Exp Biol Med* 1994; **206**: 329-344
4 **Palmer RM**, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988; **333**: 664-666
5 **Groszmann RJ**. Hyperdynamic circulation of liver disease 40 years later: pathophysiology and clinical consequences. *Hepatology* 1994; **20**: 1359-1363
6 **Lockwood AH**, Yap EW, Wong WH. Cerebral ammonia metabolism in patients with severe liver disease and minimal hepatic encephalopathy. *J Cereb Blood Flow Metab* 1991; **11**: 337-341
7 **Janigro D**, West GA, Nguyen TS, Winn HR. Regulation of blood-brain barrier endothelial cells by nitric oxide. *Circ Res* 1994; **75**: 528-538
8 **Jaworowicz DJ**, Korytko PJ, Singh Lakhman S, Boje KM. Nitric oxide and prostaglandin E_2 formation parallels blood-brain barrier disruption in an experimental rat model of bacterial meningitis. *Brain Res Bull* 1998; **46**: 541-546
9 **Smith WL**. Prostaglandin biosynthesis and its compartmentation in vascular smooth muscle and endothelial cells. *Annu Rev Physiol* 1986; **48**: 251-262
10 **Pizcueta P**, Piqué JM, Fernandéz M, Bosch J, Rodés J, Whittle BJ, Moncada S. Modulation of the hyperdynamic circulation of cirrhotic rats by nitric oxide inhibition. *Gastroenterology* 1992; **103**: 1909-1915
11 **Lee FY**, Colombato LA, Albillos A, Groszmann RJ. N omega-nitro-L-arginine administration corrects peripheral vasodilation and systemic capillary hypotension and ameliorates plasma volume expansion and sodium retention in portal hypertensive rats. *Hepatology* 1993; **17**: 84-90
12 **Sitzmann JV**, Bulkley GB, Mitchell MC, Campbell K. Role of prostacyclin in the splanchnic hyperemia contributing to portal hypertension. *Ann Surg* 1989; **209**: 322-327
13 **Bruix J**, Bosch J, Kravetz D, Mastai R, Rodéz J. Effects of prostaglandin inhibition on systemic and hepatic hemodynamics in patients with cirrhosis of the liver. *Gastroenterology* 1985; **88**: 430-435
14 **Chu CJ**, Wang SS, Lee FY, Chang FY, Lin HC, Hou MC, Chan CC, Wu SL, Chen CT, Huang HC, Lee SD. Detrimental effects of nitric oxide inhibition on hepatic encephalopathy in rats with thioacetamide-induced fulminant hepatic failure. *Eur J Clin Invest* 2001; **31**: 156-163
15 **Rush B**, Merritt MV, Kaluzny M, Van Schoick T, Brunden MN, Ruwart M. Studies on the mechanism of the protective action of 16,16-dimethylPGE2 in carbon tetrachloride induced acute hepatic injury in the rat. *Prostaglandins* 1986; **32**: 439-455
16 **Araki H**, Lefer AM. Cytoprotective actions of prostacyclin during hypoxia in the isolated perfused cat liver. *Am J Physiol* 1980; **238**: H176-H181
17 **Davis DC**, Potter WZ, Jollow DJ, Mitchell JR. Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. *Life Sci* 1974; **14**: 2099-2109
18 **Mizoguchi Y**, Tsutsui H, Miyajima K, Sakagami Y, Seki S, Kobayashi K, Yamamoto S, Morisawa S. The protective effects of prostaglandin E_1 in an experimental massive hepatic cell necrosis model. *Hepatology* 1987; **7**: 1184-1188
19 **Noda Y**, Hughes RD, Williams R. Effect of prostacyclin (PGI_2) and a prostaglandin analogue BW245C on galactosamine-induced hepatic necrosis. *J Hepatol* 1986; **2**: 53-64
20 **Ogawa M**, Mori T, Mori Y, Ueda S, Yoshida H, Kato I, Iesato K, Wakashin Y, Wakashin M, Okuda K. Inhibitory effects of prostaglandin E1 on T-cell mediated cytotoxicity against iso-

lated mouse liver cells. *Gastroenterology* 1988; **94**: 1024-1030
21 **Alp MH**, Hickman R. The effect of prostaglandins, branched-chain amino acids and other drugs on the outcome of experimental acute porcine hepatic failure. *J Hepatol* 1987; **4**: 99-107
22 **Guarner F**, Boughton-Smith NK, Blackwell GJ, Moncada S. Reduction by prostacyclin of acetaminophen-induced liver toxicity in the mouse. *Hepatology* 1988; **8**: 248-253
23 **Masaki N**, Ohta Y, Shirataki H, Ogata I, Hayashi S, Yamada S, Hirata K, Nagoshi S, Mochida S, Tomiya T. Hepatocyte membrane stabilization by prostaglandins E_1 and E_2: favorable effects on rat liver injury. *Gastroenterology* 1992; **102**: 572-576
24 **Hou MC**, Cahill PA, Zhang S, Wang YN, Hendrickson RJ, Redmond EM, Sitzmann JV. Enhanced cyclooxygenase-1 expression within the superior mesenteric artery of portal hypertensive rats: role in the hyperdynamic circulation. *Hepatology* 1998; **27**: 20-27
25 **Albrecht J**, Hilgier W, Rafalowska U. Activation of arginine metabolism to glutamate in rat brain synaptosomes in thioacetamide-induced hepatic encephalopathy: an adaptive response? *J Neurosci Res* 1990; **25**: 125-130
26 **Osada J**, Aylagas H, Miro-Obradors MJ, Arce C, Palacios-Alaiz E, Cascales M. Effect of acute thioacetamide administration on rat brain phospholipid metabolism. *Neurochem Res* 1990; **15**: 927-931
27 **Steindl P**, Püspök A, Druml W, Ferenci P. Beneficial effect of pharmacological modulation of the $GABA_A$-benzodiazepine receptor on hepatic encephalopathy in the rat: comparison with uremic encephalopathy. *Hepatology* 1991; **14**: 963-968
28 **Bengtsson F**, Gage FH, Jeppsson B, Nobin A, Rosengren E. Brain monoamine metabolism and behavior in portacaval shunted rats. *Exp Neurol* 1985; **90**: 21-35
29 **Ribeiro J**, Nordlinger B, Ballet F, Cynober L, Coudray-Lucas C, Baudrimont M, Legendre C, Delelo R, Panis Y. Intrasplenic hepatocellular transplantation corrects hepatic encephalopathy in portacaval–shunted rats. *Hepatology* 1992; **15**: 12-18
30 **Chu CJ**, Lee FY, Wang SS, Chang FY, Lin HC, Wu SL, Chan CC, Tsai YT, Lee SD. Establishment of an animal model of hepatic encephalopathy. *J Chin Med Assoc* 2000; **63**: 263-269
31 **Gammal SH**, Basile AS, Geller D, Skolnick P, Jones EA. Reversal of the behavioral and electrophysiological abnormalities of an animal model of hepatic encephalopathy by benzodiazepine receptor ligands. *Hepatology* 1990; **11**: 371-378
32 **Chu CJ**, Lee FY, Wang SS, Lu RH, Tsai YT, Lin HC, Hou MC, Chan CC, Lee SD. Hyperdynamic circulation of cirrhotic rats with ascites: role of endotoxin, tumour necrosis factor-a and nitric oxide. *Clin Sci* 1997; **93**: 219-225
33 **Needleman P**, Turk J, Jakschik BA, Morrison AR, Lefkowith JB. Arachidonic acid metabolism. *Annu Rev Biochem* 1986; **55**: 69-102
34 **Iwai M**, Jungermann K. Leukotrienes increase glucose and lactate output decrease flow in perfused rat liver. *Biochem Biophys Res Commun* 1988; **151**: 283-290
35 **Busam KJ**, Bauer TM, Bauer J, Gerok W, Decker K. Interleukin-6 release by rat liver macrophages. *J Hepatol* 1990; **11**: 367-373
36 **Nagai H**, Aoki M, Shimazawa T, Yakuo I, Koda A, Kasahara M. Effect of OKY-046 and ONO-3708 on liver injury in mice. *Jpn J Pharmacol* 1989; **51**: 191-197
37 **Matsukawa R**, Takiguchi H. Effect of indomethacin on Ca2+-stimulated adenosine triphosphatase in the synaptic vesicles of rat brain *in vitro*. *Int J Biochem* 1982; **14**: 713-717
38 **Hemker DP**, Aiken JW. Actions of indomethacin and prostaglandins E2 and D2 on nerve transmission in the nictitating membrane of the cat. *Prostaglandins* 1981; **22**: 599-611
39 **Theodorsson E**, Mathe AA, Stenfors C. Brain neuropeptides: tachykinins, neuropeptide Y, neurotensin and vasoactive intestinal polypeptide in the rat brain: modifications by ECT and indomethacin. *Prog Neuropsychopharmacol Biol Psychiatry* 1990; **14**: 387-407
40 **Old LJ**. Tumor necrosis factor (TNF). *Science* 1985; **230**: 630-632
41 **Andus T**, Bauer J, Gerok W. Effects of cytokines on the liver. *Hepatology* 1991; **13**: 364-375
42 **FitzGerald GA**, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. *N Engl J Med* 2001; **345**: 433-442
43 **Cannon GW**, Caldwell JR, Holt P, McLean B, Seidenberg B, Bolognese J, Ehrich E, Mukhopadhyay S, Daniels B. Rofecoxib, a specific inhibitor of cyclooxygenase-2, with clinical efficacy comparable with that of diclofenac sodium: results of a one-year, randomized, clinical trial in patients with osteoarthritis of the knee and hip. Rofecoxib Phase III Protocol 035 Study Group. *Arthritis Rheum* 2000; **43**: 978-987
44 **Clemett D**, Goa KL. Celecoxib: a review of its use in osteoarthritis, rheumatoid arthritis and acute pain. *Drugs* 2000; **59**: 957-980
45 **Day R**, Morrison B, Luza A, Castaneda O, Strusberg A, Nahir M, Helgetveit KB, Kress B, Daniels B, Bolognese J, Krupa D, Seidenberg B, Ehrich E. A randomized trial of the efficacy and tolerability of the COX-2 inhibitor rofecoxib *vs* ibuprofen in patients with osteoarthritis. Rofecoxib/Ibuprofen Comparator Study Group. *Arch Intern Med* 2000; **160**: 1781-1787
46 **Sinclair SB**, Greig PD, Blendis LM, Abecassis M, Roberts EA, Phillips MJ, Cameron R, Levy GA. Biochemical and clinical response of fulminant viral hepatitis to administration of prostaglandin E. A preliminary report. *J Clin Invest* 1989; **84**; 1063-1069

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