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Short Communication

Oxidative stress and hippocampus in a low-grade hepatic encephalopathy model: protective effects of curcumin

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Aim: The present study was performed on prehepatic portal hypertensive rats, a model of low-grade hepatic encephalopathy, designed to evaluate whether oxidative stress was a possible pathway implicated in hippocampal damage and if so, the effect of an anti-oxidant to prevent it.

Methods: Prehepatic portal hypertension was induced by a regulated portal vein stricture. Oxidative stress was investigated by assessing related biochemical parameters in rat hippocampus. The effect of the anti-oxidant curcumin, administered in a single i.p. dose of 100 mg/kg on the seventh, ninth and eleventh days after surgery, was evaluated.

Results: Oxidative stress in the rat hippocampal area was documented. Curcumin significantly decreased tissue malondialdehyde levels and significantly increased glutathione peroxidase, catalase and superoxide dismutase activities in the hippocampal tissue of portal hypertensive rats.

Conclusion: Oxidative stress was found to be implicated in the hippocampal damage and curcumin protected against this oxidative stress in low-grade hepatic encephalopathic rats. These protective effects may be attributed to its anti-oxidant properties.

Key words: hippocampus, curcumin, low grade encephalopathy, oxidative stress

INTRODUCTION

ACUTE AND CHRONIC liver diseases are among the most challenging tasks in gastroenterology. One of the reasons for this is the complication of the primary disease process in the liver by numerous metabolic disturbances and the accumulation of toxic products, among them ammonia.[1,2]

Hepatic encephalopathy (HE) constitutes one of the most intriguing complications in acute and chronic liver pathology. In addition to clinically manifest HE, a subclinical stage has also been described.[3] A working party report has classified HE into groups (A, B and C).[4] Experimental prehepatic portal hypertension (PPH) induced by portal vein stricture can be regarded as a valid model for the study of a subclinical or low grade HE (lgHE).[5,6]

Hyperammonemia is recognized in humans and animal models of liver disease, and has been associated with mitochondrial damage, energy impairment and increased free radical (FR) formation.[7–10]

The brain is absolutely dependent upon oxidative metabolism for cell survival and is particularly sensitive to oxidative damage because of its high content of iron, polyunsaturated fatty acids, catecholamines and excitatory amino acids, all of which may mediate oxidative stress (OS) and reactive oxygen species (ROS) production.[11–13]

Oxidative stress has emerged as a potentially important factor in the pathogenesis of HE.[14] Ammonia has been shown to generate FR *in vivo* and in cultured astrocytes. Additionally, decreased activities of anti-oxidant enzymes – glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) – and increased superoxide production ($O2^-$) have been described.[15] Besides this, OS has been strongly associated with astrocyte swelling in acute liver failure (ALF),

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as well as the induction of mitochondrial transition pore in chronic liver failure.[16]

These data encouraged us to discover whether OS was implicated in the morpho-functional alterations found in the rat hippocampal area, previously reported by our laboratory,[7] in lgHE.

If lgHE is associated with an imbalance of OS and the anti-oxidant defense system then, theoretically, it would be possible to limit oxidative damage and ameliorate disease progression by supplementing anti-oxidants.[13,17]

Curcumin (CUR) from *Curcuma longa Linn* (turmeric) – *Zingiberaceae* has proved to be, in terms of modern medicine, a neuroprotective, anti-inflammatory, antitumor, renoprotective, cardioprotective, lipid-lowering agent which exhibits anti-oxidant properties.[18–26] CUR is a potent scavenger of ROS and inhibitor of lipid peroxidation and xanthine–xanthine oxidase-induced superoxide ($O2^-$) production.[26,27] The highly lipophilic character of CUR greatly enhances its brain disposition. On these basis, the present study was designed to investigate whether OS was implicated in the alterations in the hippocampal tissue of lgHE rats when assessing ROS and related biochemical parameters.

METHODS

Animals and surgical procedures

WISTAR MALE RATS (200–250 g) were housed separately and acclimatized before use under temperature-controlled (25 ± 2 °C) and light-controlled (12 h light/dark cycle) conditions. Rats were fed with standard rat chow and water *ad libitum*. After 1 week of acclimatization, rats were randomized and separated into three groups: (I) sham operated ($n = 8$); (II) PPH rats ($n = 8$); and (III) PPH rats plus CUR ($n = 8$).

Portal hypertension was induced by a calibrated stricture of the portal vein (PPVL). Rats were anaesthetized with ether, a midline abdominal incision was made and a 3–0 silk ligature was placed around the vein and snugly tied to a 20-gauge blunt-end needle placed alongside the portal vein. The needle was subsequently removed to yield a calibrated stenosis of the portal vein (groups II and III). Sham-operated rats (group I), underwent an identical procedure except that the portal vein was not stenosed.[28]

On the seventh, ninth and eleventh days after surgery, group III received a single dose of CUR (100 mg/kg i.p.)

All animal experiments were carried out in accordance with the guidelines of the National Institute of Health (USA) for the care and use of laboratory animals.[29]

Enzyme preparations and assays

Rats were anaesthetized with ether and killed 14 days after surgery by decapitation; the brain was removed and the hippocampal area dissected, excised and homogenized in a Potter–Elvehejm homogenizer using different solutions.

CAT, SOD and GSH–Px activities were determined spectrophotometrically in brain homogenates prepared in a medium consisting of 140 mmol/L KCl and 25 mmol/L potassium phosphate buffer (pH 7.4), and centrifuged at 600 g for 10 min. The supernatant, a suspension of preserved organelles, was used as homogenate. CAT activity was determined by measuring the decrease in absorbance at 240 nm, GSH–Px activity following NADPH oxidation at 340 nm and SOD activity by inhibition of adrenochrome formation rate at 480 nm.[30–32] One unit in the SOD assay is defined as the amount of enzymatic protein required to inhibit 50% of epinephrine auto-oxidation.

Lipid peroxidation

Lipid peroxidation in the liver was determined by measuring the rate of production of thiobarbituric acid reactive substances (TBARS), expressed as malondialdehyde equivalents (MDA).[33] One volume of homogenate was mixed with 0.5 volume TCA (15% w/v) and centrifuged at 2000 *g* for 10 min. The supernatant (1 ml) was mixed with 0.5 ml thiobarbituric acid (0.7% w/v) and boiled for 10 min. After cooling, sample absorbance was read spectrophotometrically at 535 nm. MDA concentration was calculated using an ε value of 1.56×105/M/cm.

Protein determination

Protein concentration was measured following Lowry *et al.*[34] using bovine serum albumin as standard.

Statistical analysis

Results are expressed as mean ± SD. The data were analyzed statistically by factorial analysis of variance (ANOVA) followed by the Neuman–Keuls' test for comparison of means. Differences were considered significant at $P < 0.05$.

RESULTS

THE HIPPOCAMPAL ANTI-OXIDANT enzyme activities showed a statistically significant decrease in PPH rats (CAT 0.018 ± 0.003; SOD 15.3 ± 2.6; GSH–Px 0.12 ± 0.02; $P < 0.01$), when compared with

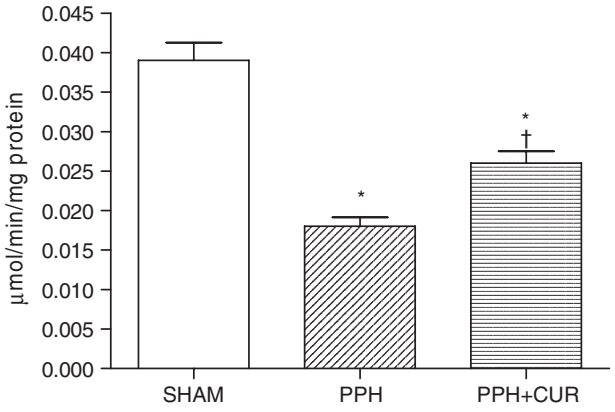

Figure 1 Catalase (CAT) activity in rat hippocampal tissue. *$P < 0.05$ versus sham group; †$P < 0.01$ versus prehepatic portal hypertension (PPH) group.

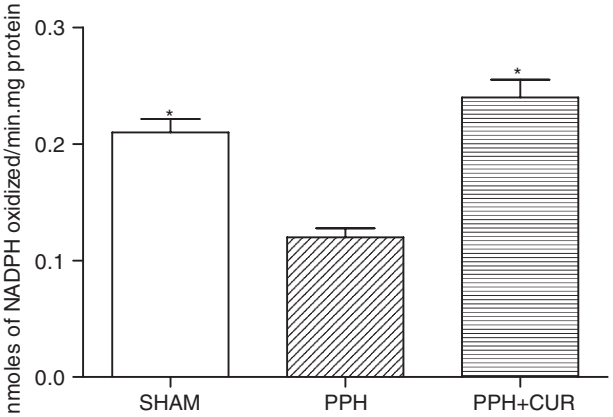

Figure 3 GSH–Px activity in rat hippocampal tissue. *$P < 0.01$ versus prehepatic portal hypertension (PPH) group.

the sham group (CAT 0.039 ± 0.006; SOD 34.2 ± 5.7; GSH-Px 0.21 ± 0.03; $P < 0.01$) (Figs 1–3).

The hippocampal tissue levels of MDA showed a statistically significant increase (Fig. 4) when compared to PPH (0.55 ± 0.09) versus the sham group (0.40 ± 0.06) ($P < 0.05$).

The administration of CUR showed a significantly increase in CAT hippocampal activity in the PPH + CUR (0.026 ± 0.004) group when compared to the PPH (0,018 ± 0,003, $P < 0.01$) and sham groups (0.039 ± 0.006, $P < 0.05$) (Fig. 1).

PPH + CUR SOD activity (24.8 ± 4.1) showed significantly increased values when compared to PPH (15.3 ± 2.6, $P < 0.01$) and similar values to the sham group (34.2 ± 5.7, not significant), see Figure 2.

PPH + CUR GSH–Px activity (0.24 ± 0.04), again showed increased significant values when compared to the PPH (0.12 ± 0.02, $P < 0.01$) group and a rise in normal values, as in the sham group (Fig. 3).

The hippocampal levels of MDA in the PPH + CUR group (0.21 ± 0.03) showed a statistically significant decrease when compared to both the sham (0.40 ± 0.06, $P < 0.01$) and PPH groups (0.55 ± 0.09, $P < 0.001$): see Figure 4.

DISCUSSION

THE PRESENT STUDY showed for the first time OS in the hippocampal area induced by PPH in lgHE rats. This was prevented by the administration of CUR. This

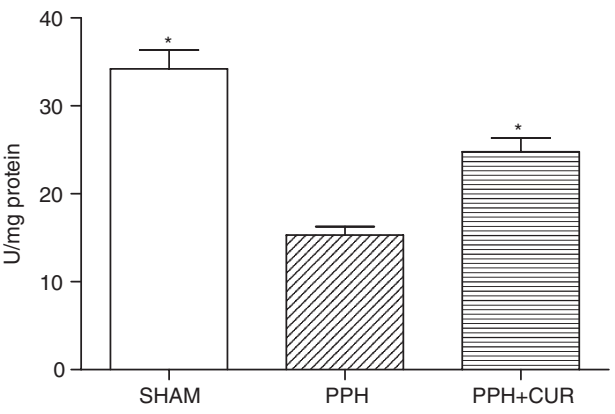

Figure 2 Superoxide dismutase (SOD) activity in rat hippocampal tissue. *$P < 0.01$ versus prehepatic portal hypertension (PPH) group.

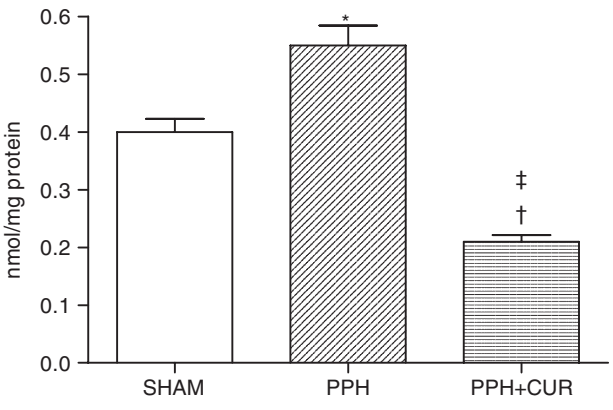

Figure 4 Malondialdehyde equivalents (MDA) levels in rat hippocampal tissue. *$P < 0.05$ and †$P < 0.01$ versus sham group; ‡$P < 0.001$ versus prehepatic portal hypertension (PPH) group.

study also suggest that hippocampal damage is directly associated with the induction of OS. In previous reports we demonstrated morphofunctional hippocampal damage in lgHE.[35] The possible role of OS in HE was originally suggested by O'Connor and Costell, who found that hyperammonemic mice displayed evidence of lipid peroxidation. Lipid peroxidation has been identified in non-synaptic mitochondria in the thioacetamide model of ALF, and cultured astrocytes treated with ammonia have also shown evidence of lipid peroxidation.[36–38] MDA, one of the major products of lipid peroxidation, has been extensively studied and measured as an index of lipid peroxidation.[39] In the present study there was an increase in hippocampal MDA level accompanied by a depletion of hippocampal GSH–Px activity (Figs 3, 4). This suggested that PPH induced overproduction of ROS, which caused hippocampal OS. Also, a significant decrease in the activities of the anti-oxidant enzymes (SOD, CAT and GSH–Px) was registered in rat hippocampal tissue (Figs 1–3).

The decrease in anti-oxidant enzyme activities may be explained as a result of an attack of ROS to the active site of the enzyme or the consumption of anti-oxidant enzymes by ROS. In fact, O_2^- inhibits CAT activity, and H_2O_2 may also inhibit SOD activity through a modification in histidine residue located in the active site of the enzyme.[40,41] Inactive or decreased SOD and CAT activities due to ROS after PPVL may lead to extensive later neuronal damage occurring in the hippocampal tissue, and may explain some of the alterations.[42–44]

Furthermore, the OS-related biochemical parameters evaluated in this study were not sufficient to indicate or to exclude the damage or protection of neurons in the absence of histological assessment, which is a reliable way to confirm neuronal damage.[45]

The administration of CUR showed preventative action against OS induced by PH. CUR has been found to prevent or reduce the oxidative stress-induced progression of Alzheimer's disease.[46,47] The activity of CUR against cytotoxicity *in vitro* and *in vivo* has also been demonstrated.[48,49]

In this study, when PPH rats were treated with CUR, the depletion of hippocampal GSH–Px activity and the increase of MDA level were prevented. This is supported by the *in vitro* experiment, which indicated that CUR protected the rat's hippocampal tissue against iron-induced lipid peroxidation at very low concentration. This anti-oxidant activity was similar to BHT (standard anti-oxidant).[13] This result indicated that CUR is a powerful anti-oxidant against lipid peroxidation induced by iron in hippocampal tissue.[45]

In biological systems, O_2^- is inactivated mainly by SOD, while H_2O_2 is decomposed to water by CAT. GSH–Px also participates in the reductive detoxification of H_2O_2. In group II, ROS could not be readily scavenged because of the low activities of SOD, CAT and GSH–Px in the brain. In the present study, the treatment markedly suppressed the declined SOD, CAT and GSH–Px activities in the hippocampal tissue.

The results of the present study indicate that OS was present and can act as a pathogenic factor in histopathological damage in the hippocampal rat area, and CUR confers protection against OS by attenuating hippocampal ROS oxidative damage in experimental lgHE.

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