BLOCKING NMDA RECEPTORS DELAYS DEATH IN RATS WITH ACUTE LIVER FAILURE BY DUAL PROTECTIVE MECHANISMS IN KIDNEY AND BRAIN

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INTRODUCTION AND AIMS

Acute liver failure (ALF) leads to rapid progression of deleterious events including systemic inflammatory response, multi-organ (including renal) failure, hyperammonemia, cerebral edema, hepatic encephalopathy (HE), increased intracranial pressure (ICP), coma, and rapid death of patients. Many patients with ALF die of increased ICP and cerebral herniation.Treatment of patients with ALF is unsatisfactory and mortality remains unacceptably high. Blocking NMDA receptors delays or prevents death of rats with ALF. The underlying mechanisms remain unclear. Clarifying these mechanisms will help to design more efficient treatments to increase patient's survival. The aim of this work was to shed light on the mechanisms by which blocking NMDA receptors delays rat's death in ALF.

MATERIALS AND METHODS

MK-801 Administration: Male Wistar rats (220–270 g) were anesthetized with isoflurane and osmotic pumps (ALZET, model 2001) releasing 1 IL per hour during 7 days were implanted subcutaneously in the back.

Two groups of rats were used:

- **VEH:** with osmotic pumps filled with vehicle (0.9 % NaCl, 200 IL)
- **MK-801:** with MK-801 (2 mg/mL) to keep NMDA receptors continuously blocked.

Induction of ALF and Neurological Evaluation: ALF was induced by i.p. injection of galactosamine (2.5g/Kg) 3 days after osmotic pumps implantation. The neurological status was assessed every 60 min, and the grades of HE were assigned. Data were pooled and expressed as grade I/II and grade III/IV.

Ammonia determination was performed as in Cauli *et al* (*Am J Physiol. 2008;295:G503-11*)

Magnetic resonance Imaging: MRI experiements were performed on a Briker Pharmascan system (Bruker Medical Gmbh, Ettlingen, Germany) using a 7.0-T horizontal bore superconducting magnet.

Lactate was measured in crebellum and frontal cortex by *in vivo* ^{1}H-MR Spectroscopy

Cerebral Blood Flow (CBF) was analyzed by MRI as in Cauli et al (*J Neurochem 2007;103:1334-43*)

Intracranial Pressure (ICP) was measured as in *Cauli et al (Gastroenterol 2011;140:638-45)*

***In vivo* Brain Microdialysis:** A microdialysis guide was implanted in cerebellum. After 48 h, microdialysis probes were introduced and perfused. 30-min samples were collected.

RESULTS

The main new findings of this study are that blocking NMDA receptors:

- **Delays the increase in ammonia in blood, muscle, cerebral cortex, and extracellular ammonia in cerebellum (Fig. 1)**
- **Delays the progression of HE, ICP, and death (Fig. 2).**
- **Prevents completely the changes in blood flow and lactate in cerebellum (Fig 3)**
- **Delays and reduces the increases in blood flow and lactate in cortex (Fig 3)**
- **Delays kidney damage and enhances ammonia elimination in urine at early stages of ALF (Fig.1E and Fig.4)**
- **ALF reduces kidney glomerular filtration rate (GFR) as reflected by reduced inuline clearance. GFR reduction is due to both reduced renal perfusion and kidney tubular damage as reflected by increased Kim-1 in urine and histological analysis. Blocking NMDA receptors delays kidney damage, allowing transient increased GFR and ammonia elimination which delays hyperammonemia and associated changes in brain. Blocking NMDA receptors does not prevent cerebral edema or blood-brain barrier permeability but reduces or prevents changes in cerebral blood flow and brain lactate.**

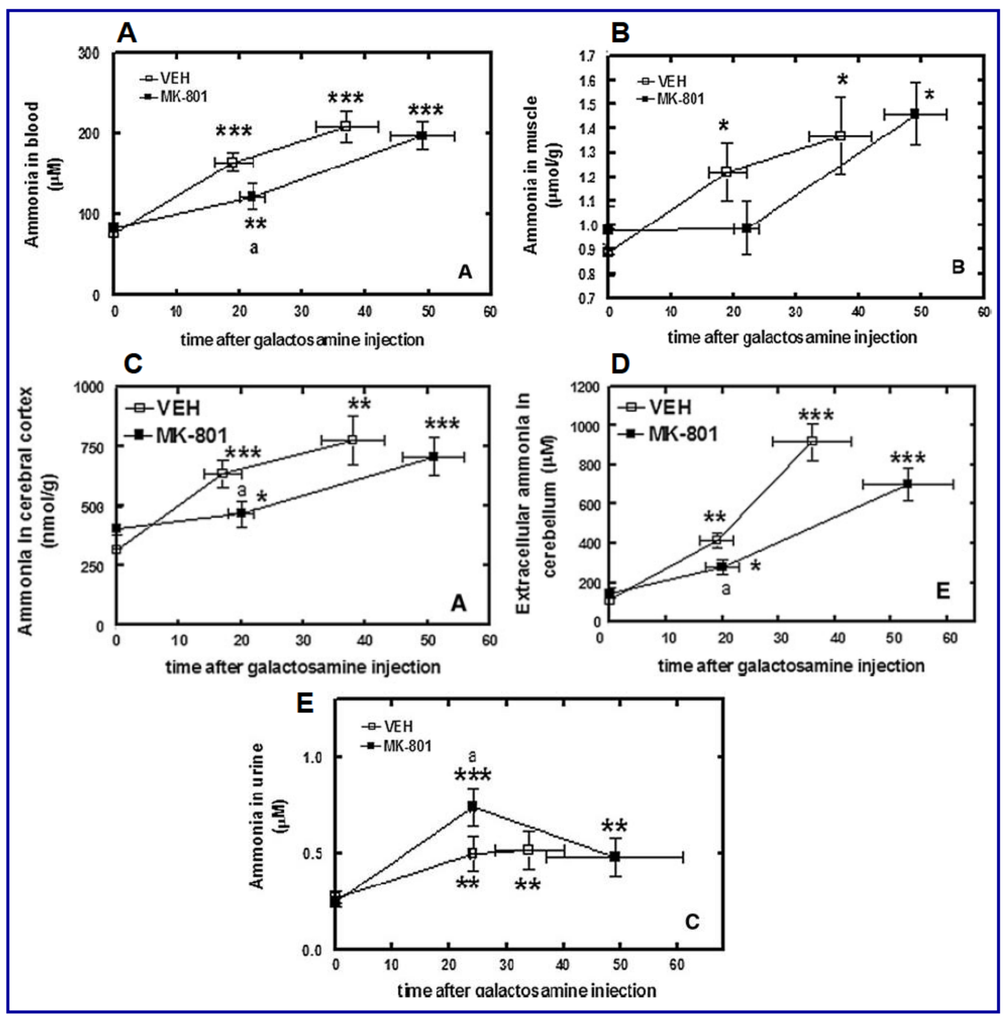

Figure 1. MK-801 delays the increase in ammonia in blood (A), muscle (B), cerebral cortex (C), and extracellular ammonia in cerebellum (D), and enhances ammonia elimination in urine (E). Ammonia was measured at different grades of HE. Values are the mean ± SEM of 6–12 rats. Values significantly different from basal are indicated by asterisks *p<0.05; **p<0.01; ***p<.001. Values significantly different in rats treated with MK-801 from controls are indicated by "a", [a]p<0.05

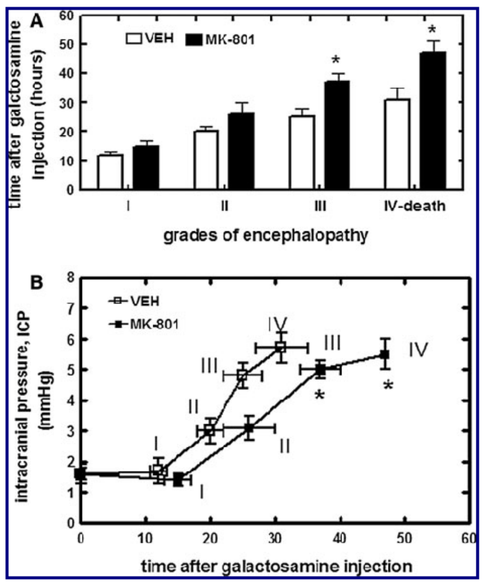

Figure 2. MK-801 delays the progression of HE, death, and ICP induced by ALF. A The **neurological status** was examined every 60 min after galactosamine injection. The time at which each grade of encephalopathy (I–IV) occurs is shown. **B ICP** in the lateral ventricle before and after galactosamine injection. Values: mean ± SEM of 8–12 rats. Values significantly different from basal values are indicated by *p<0.05; **p<0.01; ***p<0.001;Values significantly different in rats treated with MK-801 from controls are indicated by [a]p<0.05

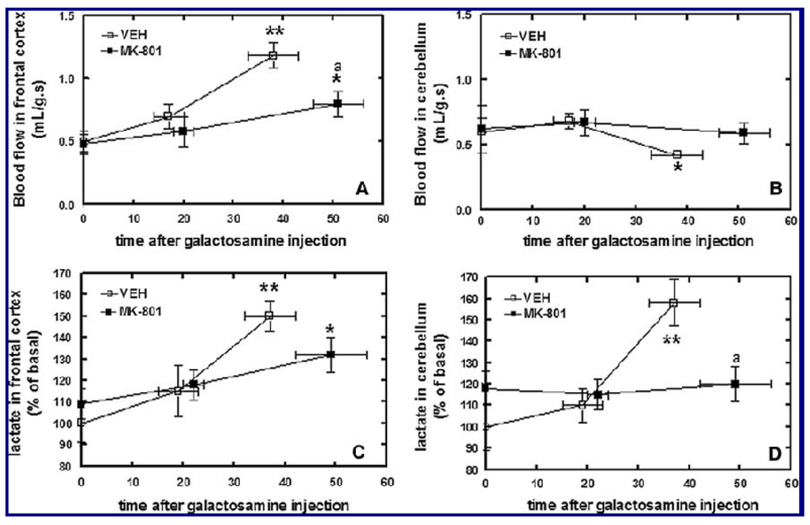

Figure 3. MK-801 delays and reduces changes in blood flow and lactate. Blood flow (a–b) and lactate (c–d) in cortex (a, c) and cerebellum (b, d) were measured at different grades of HE. Values are the mean ± SEM of 6–8 rats. Values significantly different from basal are indicated by asterisks *p\0.05; **p<0.01; Values significantly different in rats treated with MK-801 from controls are indicated by "a", [a]p<0.05

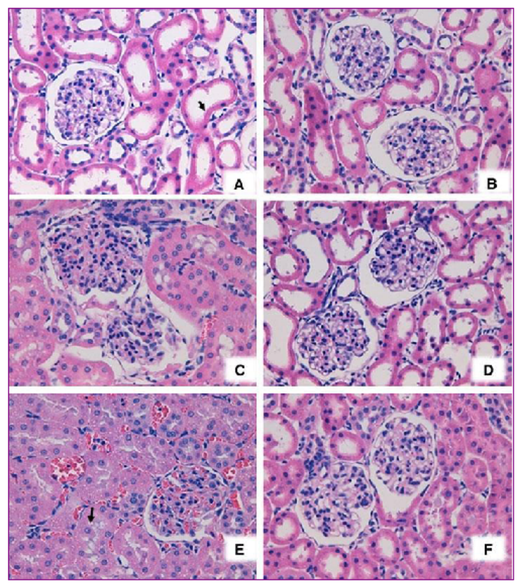

Figure 4. MK-801 delays and reduces structural tubular damage. Kidney sections were prepared and stained with hematoxylin–eosin. All images are shown at 9400 magnification. **A** Control rats treated with vehicle (arrow indicates well preserved brush border). **B** Control rats treated with MK-801. **C** Rats treated with galactosamine and killed at grade I of HE. **D** Rats treated with MK-801 and galactosamine and killed at grade I of HE. **E** Rats treated with galactosamine and killed at grade III of HE (arrow indicates vacuole). **F** Rats treated with MK-801 and galactosamine and killed at grade III of HE

CONCLUSIONS

The data show that dual protective effects of MK-801 in kidney and brain delay cerebral alterations, HE, intracranial pressure increase and death. NMDA receptors antagonists may increase survival of patients with ALF by providing additional time for liver transplantation or regeneration.

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