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Non-invasive markers of hepatic encephalopathy under chronic hepatitis C and 2-oxoglutarate treatment

Nieinwazyjne markery encefalopatii wątrobowej w przebiegu wirusowego zapalenia wątroby typu C i po podaniu 2-oksoglutaranu

INTRODUCTION

Chronic hepatitis causes progressive brain damage (hepatic encephalopathy, HE). A key role for ammonia in the pathogenesis of both HE and brain edema is now firmly supported by clinical and experimental data. The astrocytes play the key role in HE pathogenesis. The metabolism of ammonia to glutamine providion by astrocytes down regulates the osmotic disturbance and mitochondrial dysfunction with oxidative stress in the brain [4].

The main goal of our work was investigating the level of non-invasive markers of hepatic fibrosis and astroglia damage under chronic hepatitis C (CHC) and 2-oxoglutarate treatment. The non-invasive markers of chronic hepatitis C (CHC) – total protein, urea and hyaluronic acid in the blood serum are well known [2]. However, blood serum markers for astrocytes disturbance are less studied.

MATERIAL AND METHODS

The experimental CHC was developed according to patent № u2006004614 [7]. 32 Wister rats were used for the experiment according to European ethical rules. In short, carbon tetrachloride (CCL4) at a dose of 0.25 ml 50% solution in the refined oil was administered under the skin of back rat paws four times every 5 days, and after that – the complete Freund's adjuvant 0.5 ml, containing 0.5 mg of BCG and 5 mg of liver protein homogenate, was injected in the root of the rat tail; after 8 days the azathioprine was administered at a dose of 50 mg/kg, next 7 days after – complete Freund's adjuvant with liver homogenate 0.25 ml, then 5 days after – azathioprine, 4 days after – Freund's adjuvant with liver homogenates 0.25 ml. 2-oxoglutarate was given to the rats in drinking water (0.228%) during 2 weeks after CHC development.

The level of S-100b protein was measured with ELISA using monospecific polyclonal antiserum against S100b (Sigma, USA) and highly purified S100b (Sigma) as a standard. Optical density was measured with the help of Anthos-2010 absorbance reader (Anthos Labtec Instruments, Austria). The concentration of total protein was measured using the Bradford protein assay [3]. The level of urea in the blood serum was measured with Urea kit (Reagent, Ukraine), the concentration of hyaluronic acid – according to Gold methods [5]. Statistical analysis was performed using Statistica software (version 5, StatSoft, Tulsa, OK, USA). Values are shown as mean ± standard error of the mean (SEM).

RESULTS

The development of CHC induced decreased level of the total serum protein (49.9±4.0 mg/ml compared to the control 62.4±2.2 mg/ml) and urea in the blood serum (5.62±0.22 mmol/l compared to the control 7.26±0.13 mmol/l). The level of hyaluronic acid in the blood serum was increased to 2.74±0.11 μg/ml compared to the 2.1±0.07 μg/ml under CHC condition (Fig. 1). The development of CHC leads to elevation of the calcium-binding protein S-100b level (specific for astrocytes) in all studied brain regions (data not presented). The immunohistochemical data show that the number of astrocytes in the cerebellum changed in the Alzheimer type II cells was elevated. It was coincident with increased level of the S-100b in the blood serum. The treatment with 2-oxoglutarate 0.228% in drinking water 2 weeks after CHC development prevents increasing uptake of that protein to the blood and decreasing total serum protein, while the increased level of hyaluronic acid and urea were still saved in the blood.

DISCUSSION

Strong elevation (more than to four times) of S-100b concentration in the blood indicated decreased properties of blood brain barer (BBB) during chronic hepatitis C development. Brain accumulation of calcium occurs in a number of pathological conditions. Biochemical data indicated the elevation of S-100b level in cytosolic fraction of different brain regions of rats with CHC which reflects astrocyte reaction to the liver toxicity. Hepatic encephalopathy (HE) is a common reversible neuropsychiatric syndrome associated with chronic and acute liver dysfunction and significant morbidity and mortality. Although a clear pathogenesis is yet to be determined, elevated ammonia in the serum and the central nervous system are the mainstay for pathogenesis and treatment. An increased ammonia level raises the amount of glutamine within astrocytes, causing an osmotic imbalance resulting in cell swelling and ultimately brain oedema [4]. Early studies noted a significant inhibition of brain mitochondrial ketoglutarate dehydrogenase at pathological concentrations of ammonia (0.2–2 mM), possible calcium accumulation have been proposed as a deleterious effect on KGDH activity [6]. Moreover, minimal hepatic encephalopathy (MHE) is a neuro-cognitive dysfunction which occurs under hepatic disorders characterized by a specific, complex cognitive dysfunction which is independent of sleep dysfunction or problems with overall intelligence [1]. Minimal hepatic encephalopathy can have a far-reaching impact on the quality of life, and progression to overt hepatic encephalopathy. The non-invasive testing for MHE and a subsequent therapy is very important to consider this under different hepatic disorders. The level of increased concentration of S-100b in the

blood serum can help to indicate the level of risk to hepatic encephalopathy development. Effective treatment options for hepatic encephalopathy are still limited. Based on the principle that ammonia contributes to the pathogenesis of hepatic encephalopathy, current therapeutic approaches are directed at enhancing its elimination. Management includes prompt treatment of precipitating factors (infection, gastrointestinal bleeding, electrolyte disturbances, hepatocellular carcinoma, dehydration, hypotension, and the use of benzodiazepines, psychoactive drugs, and/or alcohol). Newer therapies being investigated in humans with clinical promise include 2-oxoglutarate. Since ammonia freely crosses the blood-brain barrier and astrocytes are responsible for maintaining the BBB, the presence of extra 2-oxoglutarate in the blood could produce a rapid glutamine synthesis. It could, therefore, prevent the entry of high amounts of ammonia from circulation to attenuate neurotoxicity. Our data indicate that the treatment with 2-oxoglutarate in a dose 0.228% in the drinking water during 10 days after CHC development down regulates the level of S-100b in the blood.

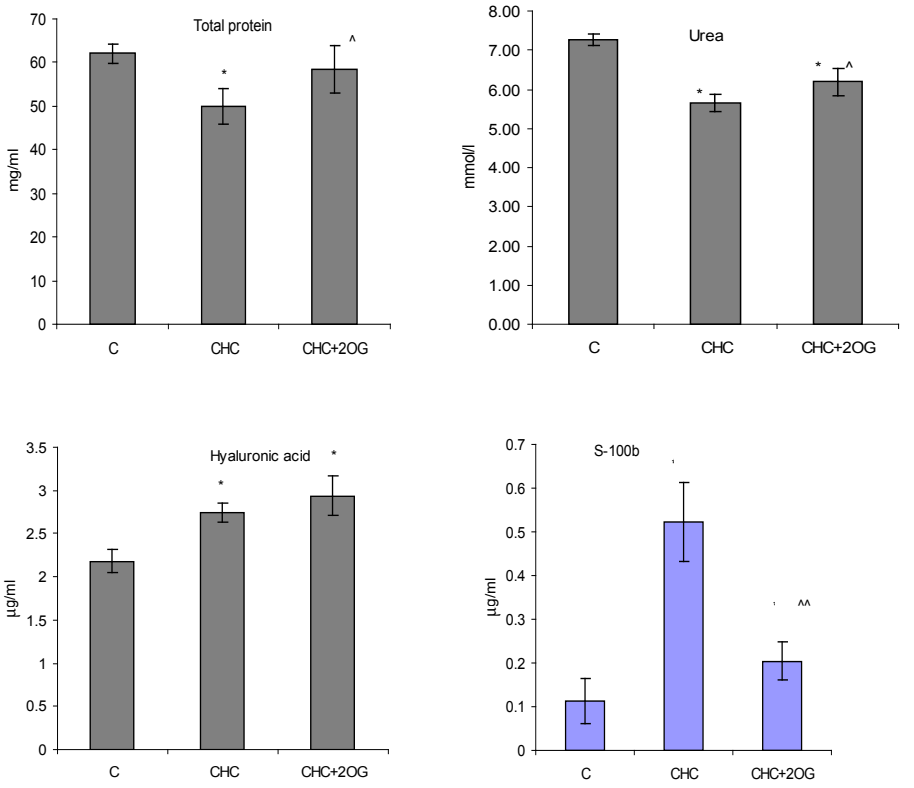

Fig. 1. The level of the non-invasive markers of liver fibrosis and astrocyte damage in the blood serum; C – control; CHC – chronic hepatitis C; CHC+2OG – treatment with 2-oxoglutarate, 0.228% in drinking water during 2 weeks after CHC development; n=6; * $p<0.001$ (compared to the control group); ^ $p<0,1$; ^^ $p<0.001$ (compared to the CHC group)

CONCLUSIONS

The obtained data allow us to suggest that the S-100b level in the blood serum may be used as a marker of astrocytes damage induced by liver toxicities under chronic hepatitis C. The treatment with 2-oxoglutarate can prevent serious brain damage.

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SUMMARY

The level of the non-invasive markers of hepatic fibrosis and astroglia damage under chronic hepatitis C (CHC) and 2-oxoglutarate treatment was investigated. The obtained data allow for the suggestion that S-100b level in the blood serum may be used as a marker of hepatic encephalopathy under CHC, the treatment with 2-oxoglutarate can prevent serious brain damage caused by ammonia effect.

Keywords: chronic hepatitis C, encephalopathy, S-100b, 2-oxoglutarate

STRESZCZENIE

Określono poziomy nieinwazyjnych markerów zwłóknienia wątroby i zniszczenia astrogleju w efekcie przewlekłego zapalenia wątroby typu C i podawania 2-oksoglutaranu. Uzyskane dane sugerują, że poziomy S-100b w surowicy krwi mogą być stosowane jako marker encefalopatii wątrobowej w przebiegu WZW typu C, zaś podanie 2-oksoglutaranu może zabezpieczyć przed poważnymi uszkodzeniami mózgu spowodowanymi działaniem amoniaku.

Słowa kluczowe: przewlekłe zapalenie wątroby typu C, encefalopatia, S-100b, 2-oksoglutaran