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A STUDY OF THE LEVELS OF GLUTATHIONE PEROXIDASE IN PATIENTS WITH SPONTANEOUS BACTERIAL PERITONITIS

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Abstract - Spontaneous bacterial peritonitis (SBP) is a major complication of liver cirrhosis, which is associated with increased mortality. While recent studies have demonstrated the involvement of reactive oxygen species in the pathogenesis of liver cirrhosis, the role of oxidative stress in the development of SBP has not yet been completely established. The present study aims to evaluate the role of oxidative stress in the pathogenesis of this complication and also the relevance of the specific treatment on these aspects. We present here some of our preliminary results regarding the specific activity of glutathione peroxidase (GPX), a very important antioxidant enzyme, from both serum and ascitic fluid of patients with decompensated cirrhosis and SBP, patients diagnosed with decompensated liver cirrhosis with ascites and patients with compensated liver cirrhosis. Our results demonstrate the presence of an increased oxidative stress in patients with decompensated cirrhosis and SBP compared with those without SBS and those with compensated liver cirrhosis, as demonstrated through the significant decrease of the specific activity of GPX. The measurement of these oxidative stress parameters may have an important role in the diagnosis and follow-up of this important liver pathology and the auxiliary treatment.

Key words: Glutathione peroxidase, cirrhosis, spontaneous bacterial peritonitis

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a major complication of liver cirrhosis, the incidence in hospitalized patients with ascitogenic cirrhosis ranging between 7% and 23%. This could be also increased by low protein concentration in ascitic fluid (below 1g/100ml), as well as by severe hepatic impairment (Stanciu et al., 2006).

In most cases, SBP is a monobacterial infection, with the culture of the ascitic fluid revealing a single microorganism and the presence of more than 250/mm^3 neutrophils. The essential pathogenic elements in SBP are intestinal bacterial overgrowth, bacterial translocation, increased intestinal permeability and immune deficiency.

The long-term prognosis of patients with SBP is poor, with the recurrence rate being 43% at 6 months. In addition, despite the use of empirical antibiotic therapy, particularly cefotaxime, as well as antibiotic prophylaxy, mortality currently ranges from 10-30% (Garcia-Tsao, 2004).

Considering that prompt diagnosis is essential in improving the prognosis of these patients, there is an urgent need for additional investigations regarding

new methods with high specificity and sensitivity, which could be used for the rapid diagnosis of this complication.

Recent studies have demonstrated the involvement of oxygen free radicals in the pathogenesis of liver cirrhosis (Irimia et al., 2013; Natarajan et al., 2006; Sakena et al., 2010; Hanafy et al., 2005), but the role of oxidative stress in the development of SBP has not yet been completely established.

We present some of our preliminary results regarding the specific activity of glutathione peroxidase, a very important antioxidant enzyme, from both serum and ascitic fluid of patients with decompensated cirrhosis and SBP, patients diagnosed with decompensated liver cirrhosis with ascites and patients with compensated liver cirrhosis.

PATIENTS AND METHODS

The study is a prospective case control that included 33 patients divided into 3 groups: group I comprised 10 patients with decompensated cirrhosis and spontaneous bacterial peritonitis (SBP); group II, 17 patients diagnosed with decompensated liver cirrhosis with ascites and group III, 6 patients with compensated liver cirrhosis.

The control group consisted of 19 healthy subjects recruited from hospital personal and matched to the patients by age and gender. SBP diagnosis was made based on clinical examination (fever, impaired general condition) and biological explorations (neutrophilic leukocytosis in blood and ascitic fluid). An essential criterion for the diagnosis of SBP was the presence of >250 neutrophils/mm^3.

Compensated cirrhosis was defined as the absence of ascites in cirrhotic patients. This presence of ascites marks the decompensated stage.

Biochemical estimations

Determination of GPX

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (SIGMA). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity.

Data analysis

The levels of GPX were statistically analyzed by using one-way analysis of variance (ANOVA). All results are expressed as mean ± SEM. F values for which $p<0.05$ was regarded as statistically significant.

RESULTS

Regarding the levels of GPX from the serum, we demonstrated a significant decrease in the patients with decompensated cirrhosis and SBP ($F(1,27)=7$, $p=0.004$), as compared to the control group (Fig. 1).

Similar decreases in the GPX specific activities were also observed in the decompensated cirrhosis without SBP-group ($F(1,30)=1$, $p=0.26$) and also in the patients with compensated liver cirrhosis ($F(1,22)=0.5$, $p=0.56$), when compared to control patients (Fig. 1).

Very importantly, when we performed the post-hoc analysis, we also observed very significant statistical differences between the group with decompensated cirrhosis and SBP vs. decompensated cirrhosis without SBP-group ($p = 0.001$), as well as between the group with decompensated cirrhosis and SBP vs. the group of patients with compensated liver cirrhosis ($p = 0.007$). However, no significant differences were observed between the group of patients with decompensated cirrhosis without SBP-group vs. the patients with compensated liver cirrhosis ($p = 0.744$) (Fig. 1).

Regarding the analysis of ascitic fluid, we also demonstrated a significant decrease in the GPX specific activity in the group with SBP, as compared with

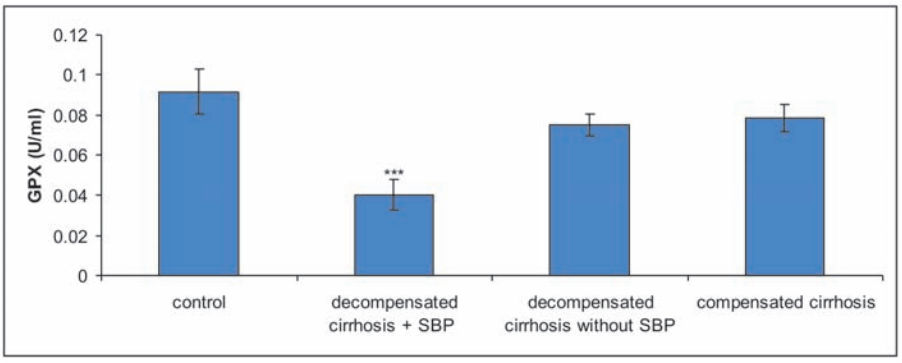

Fig. 1. Glutathione peroxidase specific activity in the serum of our research groups. The values are mean ± SEM. ***p = 0.004 vs. control group.

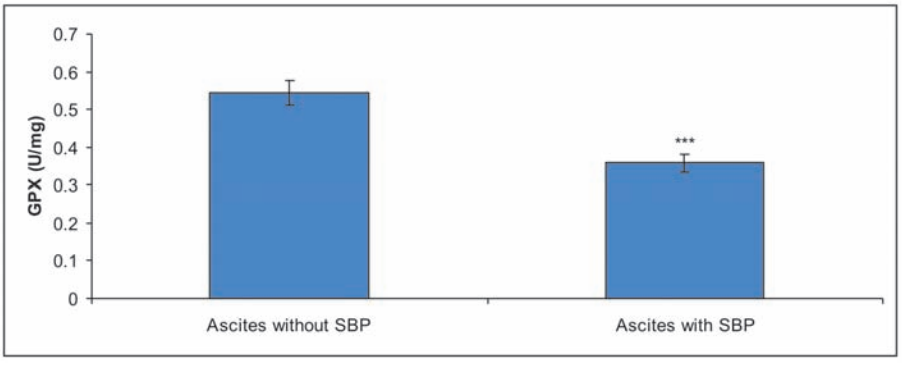

Fig. 2. Glutathione peroxidase specific activity in the ascitic fluid of our research groups. The values are mean ± SEM. ***p = 0.0001 vs. ascites without SBP.

the patients without SBP (F(1,20)=22, p=0.00015) (Fig. 2).

DISCUSSION

As previously mentioned, very few recent studies describe the very important role of oxidative stress in the pathogenesis of SBP. One important possible mechanism could be represented by an increased intestinal permeability, an essential element in SBP. As an example, it was evidenced that in these patients there is a significant increase in the processes of lipid peroxidation, completed with various alteration of the enterocytic mitochondrial function (Misra et al., 1997).

Additionally, an important role is played by endotoxinemy and the increased levels of proinflammatory cytokines and nitric oxide, which may mediate the intestinal hyperpermeability, bacterial overgrowth and the immune response (Guarner et al., 1993).

Generally, oxidative stress is defined as an imbalance between the pro-oxidant and antioxidant mechanisms in the human body. While oxidative stress is involved in the genesis of various diseases with high prevalence in modern medicine (Halliwel and Gutteridge, 2007; Ciobica et al., 2011, 2012; Padurariu et al., 2013; Stefanescu et al., 2012), recent studies have demonstrated the involvement of oxidative stress in liver pathology as well (Irimia et al., 2013; Natarajan et al., 2006; Sakena et al., 2010; Hanafy et al., 2005). The liver plays a vital role in the metabolism of different toxic substances entering the human body, being one of the organs with significant antioxidant capac-

ity. In this context, there is a growing interest in evaluating the effects and mechanisms of oxidative stress in liver pathology and, on the other hand, identifying ways to reduce these adverse effects and finding new substances with a hepatoprotective role.

The implication of oxidative stress in liver pathology was demonstrated by studying various biochemical pathways in which oxidative stress can cause lipid peroxidation of cell membranes, lesions in DNA and oxidative modifications of proteins. Therefore, reducing oxidative stress could be reflected in the diminishing of liver damage severity (Poli et al., 1997).

Additionally, previous studies have shown a correlation between hepatic and plasma glutathione (a tripeptide synthesized in the liver, which acts as an antioxidant) levels (Shigesawa et al., 1992). In addition, it has been shown that erythrocytic antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), are affected in liver diseases, in particular those which have progressed to cirrhosis. Lipoperoxide concentration, which could be translated as an increased production of free radicals, has also been demonstrated to be higher in cirrhotic patients, as compared to normal people (Gerli et al., 1992).

A very recent study also demonstrated the role of oxidative stress in the worsening of the severity of cirrhosis, assessed by Child-Pugh score, by determining the levels of pro-oxidant substances (such as serum levels of malonildialdehide (MDA)) and antioxidants (such as superoxide dismutase and glutathione peroxidase) markers in cirrhotic patients (Bhandari et al., 2008). A recent study published by Shaden et al. (2009) demonstrated the involvement of oxidative stress in SBP. In this case, oxidative stress was evidenced by elevated concentrations of MDA (an important marker of lipid peroxidation processes) and by significantly reduced activity of SOD, GPX and catalase (antioxidant components) at the serum level. In addition, an increased concentration of nitric oxide (NO) was demonstrated in patients with liver cirrhosis complicated with SBP, but not in patients with sterile ascites. This could be extremely important, considering that NO plays a very important part in the possible correlations that might exist between oxidative and nitrosative stress in generating various pathological alterations (Bild et al., 2013). It was demonstrated that after antibiotic treatment, the expression of MDA, NO and TNF-α is significantly decreased, which is also compensated by a significant increase in the antioxidant elements (Shaden et al., 2009). The Rodríguez group have shown that SBP was associated with increased levels of proinflammatory cytokines (such as IL-1, IL-6 and TNF-α) in the ascitic fluid, as compared with controls without liver cirrhosis. Moreover, the levels of proinflammatory cytokines in the ascitic fluid decrease rapidly after eradication of the infection (Rodríguez et al., 2001).

CONCLUSIONS

Our results show the presence of an increased oxidative stress in patients with decompensated cirrhosis and SBP, as compared with those without SBP and those with compensated liver cirrhosis, which is demonstrated through the significant decrease in the specific activity of GPX, a very important antioxidant enzyme. In this way, the measurement of these oxidative stress parameters may have an important role in the diagnosis and follow-up of this important liver pathology and the auxiliary treatment.

REFERENCES

Bhandari S., Mukul P.A., Dwivedi S. and *B.D. Banerjee* (2008). Monitoring oxidative stress across worsening Child Pugh class of cirrhosis. *Indian J Med Sci.* **62**, 444-451.

Bild, W., Ciobica, A., Padurariu, M. and *V Bild* (2013). The interdependence of the reactive species of oxygen, nitrogen and carbon. *Journal of Physiology and Biochemistry*. **69**, 147-154.

Ciobica, A., Olteanu, Z., Padurariu, M. and *L. Hritcu* (2012). The effects of low-dose pergolide on memory and oxidative stress in a 6-OHDA induced rat model of Parkinson's disease. *Journal of Physiology and Biochemistry*. **68**, 59-69.

Ciobica, A., Padurariu, M., Dobrin, I., Stefanescu, C. and *R. Dobrin* (2011). Oxidative stress in schizophrenia – focusing on the main markers. *Psychiatr Danub.* **23**, 237-45.

Ciobica, A., Bild, V., Hritcu, L., Padurariu, M. and *W. Bild* (2011). Effects of angiotensin II receptor antagonists on anxiety and some oxidative stress markers in rat. *Central European Journal of Medicine*. **6**, 331-340.

Garcia-Tsao, G. (2004). Spontaneous bacterial peritonitis: a historical perspective. *J Hepatol.* **41**, 522-7.

Gerli, G., Locatelli, G.F., Mongiat, R., Zenoni, L., Agostoni, A. and *G. Moschini* (1992). Erythrocyte antioxidant activity, serum ceruloplasmin and trace element levels in subjects with alcoholic liver disease. *Am J Clin Path.* **97**, 614-8.

Guarner, C., Soriano, G., Tomas, A., Bulbena, O., Novella, M.T. and *J. Balanzo* (1993) .Increased serum nitrite and nitrate levels in patients with cirrhosis: relationship to endotoxemia. *Hepatology.* **18**, 1139-1143.

Halliwell, B. and *Gutteridge, J.M.C.* (2007). Free radical in biology and medicine, 4th edn. Oxford Univ Press, New York.

Hanafy, S., El.Sayed, I.H., Mostafa, E., Gamal, A. and *F.Z. Mohamed* (2009). Oxidative Stress and Antioxidant Defence in Cirrhotic Patients Associated with Spontaneous Bacterial Peritonitis. *Journal of Applied Sciences Research*. **5**, 1785-1795.

Irimia, R., Ciobica, A., Stanciu, C. and *A. Trifan* (2013). The relevance of oxidative stress in cirrhotic patients with different forms of hepatic encephalopathy**.** *Arch. Biol. Sci., Belgrade.* **65**.

Misra, V., Misra, S., Dwivedi, M. and *S. Gupta* (1997). Histomorphometric study of portal hypertensive enteropathy. *Am J Clin Pathol.* **108**, 652-657.

Natarajan, S.K., Thomas, S., Ramamoorthy, P., Basivireddy, J., Pulimood, A.B., Ramachandran, A. and K.A. Balasubramanian (2006). Oxidative stress in the development of liver cirrhosis: a comparison of two different experimental models. *J Gastroenterol Hepatol*. **21**, 947-57.

Padurariu, M., Ciobica, A., Lefter, R., Lacramioara Serban, I., Stefanescu, C. and *R. Chirita* (2013). The oxidative stress hypothesis in Alzheimer's disease. *Psychiatr Danub*.

Poli, G. and *M. Parola* (1997). Oxidative damage and fibrogenesis. *Free Rad Biol Med.* **22**, 287-305.

Rodríguez-Ramos, C., Galan, F., Díaz, F., Elvira, J., Martín-Herrera, L. and *J.A. Girón-González* (2001). Expression of proinflammatory cytokines and their inhibitors during the course of spontaneous bacterial peritonitis. *Dig Dis Sci.* **46**, 1668-76.

Sakena, H.R., Fatima, A.M. and *M.K. Saeid* (2010). Antioxidant status and some biochemical parameters in cirrhotic liver patients. *National Journal of Chemistry.* **40**, 742-751.

Shaden, M.H., El Sayed, I.H., Mostafa, M.E., Badra, G.A. and *F.Z. Mohamed* (2009). Oxidative Stress and Antioxidant Defence in Cirrhotic Patients Associated with Spontaneous Bacterial Peritonitis**.** *Journal of Applied Sciences Research*. **5**, 1785-1795.

Shigesawa, T., Sato, C. and *F. Marumo* (1992). Significance of plasma glutathione measurements in patients with alcoholic and non-alcoholic liver disease. *J Gastroenterol Hepatol*. **7**, 7-11.

Stanciu, C., Dimache, M. and *C. Anton* (2006). Peritonita bacteriană spontană în Esentialul în hipertensiunea portală, *Editura Junimea*, 129-142.

Stefanescu, C. and *A. Ciobica* (2012). The relevance of oxidative stress status in first episode and recurrent depression, *Journal of Affective Disorders*. **143**, 34-8.