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Effect of IGF-I on total serum antioxidant status in cirrhotic rats

The role of free radical generation and oxidant injury in the pathogenesis of liver injury and fibrosis is now well established (3, 4, 9-11, 14). Our previous results showed that the enzymatic antioxidants are reduced in animals with CCl_4- induced liver cirrhosis (1).

Insulin-like growth factor–I (IGF-I) is an anabolic hormone produced mainly by the liver (12, 13). In cirrhosis a progressive fall of serum IGF-I levels is observed (2, 5, 6, 8, 9). The exogenous administration of IGF-I was able to increase the hepatic levels of antioxidant enzymes and to reduce the levels of lipid peroxidation and fibrogenesis (1, 3, 9). The aim of the present study was to analyze serum total antioxidant capacity in rats with compensated or advanced cirrhosis either in untreated or treated with low doses of IGF-I.

All experimental procedures were performed in accordance with The Guiding Principles for Research Involving Animals. Cirrhosis was induced as previously described (1), following two protocols: *Protocol A*, included male Wistar rats with early CCl_4 -induced cirrhosis (11 weeks of CCl_4 inhalation) divided into two groups: CI, untreated cirrhotic rats and CI+IGF, cirrhotic rats treated with IGF-I (2 μg x 100^{-1} x day $^{-1}$) for 2 weeks (n=10, each group). *Protocol B*, included male Wistar rats with advanced CCl_4 induced-cirrhosis and ascites (30 weeks of CCl_4 inhalation) divided into two groups: CI, untreated cirrhotic rats and CI+IGF, cirrhotic rats treated with IGF-I (2 μg x 100^{-1} x day $^{-1}$) during three weeks (n=10 for each group). In both protocols, age and sex-matched healthy control rats were included (CO, n=10).

At the end of the treatment, blood samples were drawn from the retroocular venous plexus from all the rats with capillary tubes (Marienfeld, Germany) and stored at –20 °C. After decapitation of animals, the liver was dissected and weighed. A sample from the left major liver lobe was processed for histological examination (fixed in Bouin´s solution). Sections (4 μm) were stained with haematoxylin and eosin and Masson's trichrome.

Serum total antioxidant status was evaluated using a colorimetric assay (Randox Laboratories Ltd, Ardmore, Crumlin, UK) using the following principle: Abts (2,2´-Azino-di- [3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $Abts^{\bullet+}$. This has a relatively stable blue-green colour, which is measured at 600nm. Antioxidants in the added sample cause suppression of this colour production to a degree that is proportional to their concentration (6).

Liver cirrhosis was histologically validated in all animals treated with CCl_4 and ascites were observed in all cirrhotic rats included in the Protocol B.

In the protocol A, serum total antioxidant status (TAS) was diminished in animals with compensated cirrhosis compared to controls (CI= 1.04±0.02; CO= 1.35±0.03 mmol/L, $p<0.01$) and a significant recovery was found in serum from IGF-I treated-cirrhotic rats (CI+IGF= 1.15±0.14) as compared to untreated cirrhotic group ($p<0.05$) (Fig. 1,A).

In the protocol B, the TAS was significantly reduced in serum from untreated cirrhotic rats (CI= 1.03±0.02 mmol/L) as compared with controls (CO= 1.13±0.02). In the CI+IGF group the TAS was not significantly higher (1.09±0.02) than in the untreated cirrhotic animals, and no significant differences were found between both cirrhotic groups (Fig. 1,B).

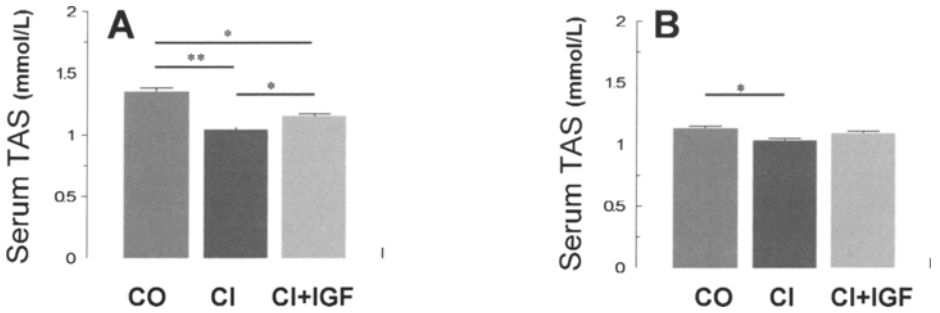

Fig. 1. *Serum total antioxidant status (TAS, as mmol/L) in healthy controls (CO), untreated cirrhotic animals (CI) and IGF-I-treated cirrhotic rats (CI+IGF).*
A corresponds to the Protocol A, animals with compensated cirrhosis, CI+IGF group was treated with low doses of IGF-I during 2 weeks. B, Protocol B, animals with advanced cirrhosis and ascites, and IGF-I treatment during 3 weeks.Values are mean ± SEM, n=10 each group, *p<0.05 and **p<0.01.

In summary, the total antioxidant status is reduced in serum from animals with liver cirrhosis including those with compensated cirrhosis and the exogenous administration of low doses of IGF-I increases serum antioxidant capacity. These data provide new evidence of the beneficial effect of IGF-I supplementation in experimental liver cirrhosis.

Key words: Oxidative stress, Antioxidants, Total antioxidant status, Liver cirrhosis, IGF-I.

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