

RELATIONSHIP BETWEEN SERUM TRANSFORMING GROWTH FACTOR β 1 AND LIVER COLLAGEN CONTENT IN RATS TREATED WITH CARBON TETRACHLORIDE

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ABSTRACT – *Context* - Transforming Growth Factor β 1 (TGF β 1) plays a fundamental role in fibrogenesis, although its importance as a biomarker of liver disease is still matter of debate. *Objective* - Quantify serum TGF β 1 and its association to liver collagen content in rats exposed to Carbon Tetrachloride (CCl₄). *Methods* - Rats were submitted to a fibrosis model using CCl₄ and sacrificed after 6, 10, 12 and 16 weeks of treatment. Serum levels of TGF β 1 were measured by ELISA and collagen content was defined by morphometric analysis. *Results* - Serum levels of TGF β 1 increased between 6 and 10 weeks, whereas collagen density increased between 12 and 16 weeks. A negative correlation was observed between liver collagen deposition and serum levels of TGF β 1 ($r = -0.48$; $P < 0.05$). *Conclusion* - Serum levels of TGF β 1 were inversely proportional to collagen intensity in cirrhotic livers of rats exposed to CCl₄, thus suggesting a limited use as biomarker in advanced liver disease.

HEADINGS – Liver cirrhosis, experimental. Transforming growth factor beta 1. Collagen. Carbon Tetrachloride. Rats.

INTRODUCTION

Liver fibrosis may be triggered by factors such as viruses, extra hepatic billiary obstruction, toxins and metabolic syndrome. All chronic liver diseases have in common the gradual progressive substitution of the functioning hepatic parenchyma by fibrotic tissue, which can evolve to cirrhosis⁽⁶⁾. Currently, the mechanisms of fibrogenesis are under extensive study, due to the increase in obesity and the high prevalence of hepatitis C virus⁽⁶⁾. Although the histopathologic study of the liver has been considered the “gold standard” for grading and staging fibrosis, liver biopsy has disadvantages such as sampling error, inter-observer variability, potential complications and its accuracy nowadays is questioned. There has been increasing interest in non-invasive assessment of liver fibrosis in place of liver biopsy using surrogate markers measurable in the peripheral blood or by using instrumental devices^(11, 14). The serological noninvasive markers of liver fibrosis can be classified into indirect (or surrogate) and direct (or biomarkers). Among direct tests hyaluronic acid,

procollagens, matrix metalloproteins and cytokines such as TGF β 1 and adiponectin are used⁽⁶⁾.

Transforming Growth Factor β 1 (TGF β 1) is one of the main profibrotic cytokine involved in liver fibrosis, inducing differentiation of hepatic stellar cells (HSCs) into myofibroblasts⁽⁹⁾. The activation of HSC leads to increased production of collagen, proteoglycans, structural glycoproteins, and hyaluronic acid⁽⁷⁾. Therefore TGF β 1 has been considered a target for treatment of fibrosis, as wells as a useful biomarker for staging liver disease^(1, 10, 4).

In this study we aimed to evaluate serum levels of TGF β 1 in rats submitted to experimental Carbon Tetrachloride (CCl₄) toxicity model and its correlation with collagen deposition in liver tissue.

METHODS

Male Wistar rats ($n = 25$) were submitted to CCl₄ administration as described by Goldani et al.⁽⁵⁾. Animals were sacrificed at weeks 6, 10, 12 and 16 ($n = 5$ per time point). Before sacrifice in CO₂ chambers, blood was collected and kept at -80°C until analysis. Serum TGF β 1 measurement was performed by ELI-

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SA (R&D Systems, USA), according to the manufacturer's instructions. Basal levels were determined in five non-exposed animals. After sacrifice, liver was paraffin-embedded and Sirius red stained. Collagen density was defined as the mean values of collagen percentage in five liver images, quantified by the morphometric method, as described by de Oliveira et al.⁽³⁾. Image analysis was made with Image-Pro Plus version 4.1 software (Media Cybernetics, USA). Statistical analysis was performed with Microsoft Excell 2007 (Microsoft Corp, USA) and SPSS 15.0 (SPSS Inc, USA) Findings were expressed as mean \pm SD and compared using Student's test or analysis of variance followed by the Tukey post hoc test. Correlations were evaluated using Pearson's test.

RESULTS

Serum levels of TGF $\beta 1$ increased around 6 times after 6 and 10 weeks of CCl₄ exposure (Figure 1). Meanwhile, liver collagen deposition was still low with a gradual increase from week 6 to 10. However, a steady increase was observed between weeks 10 and 12, with the percentage of collagen reaching almost 50% at week 16. During this same period

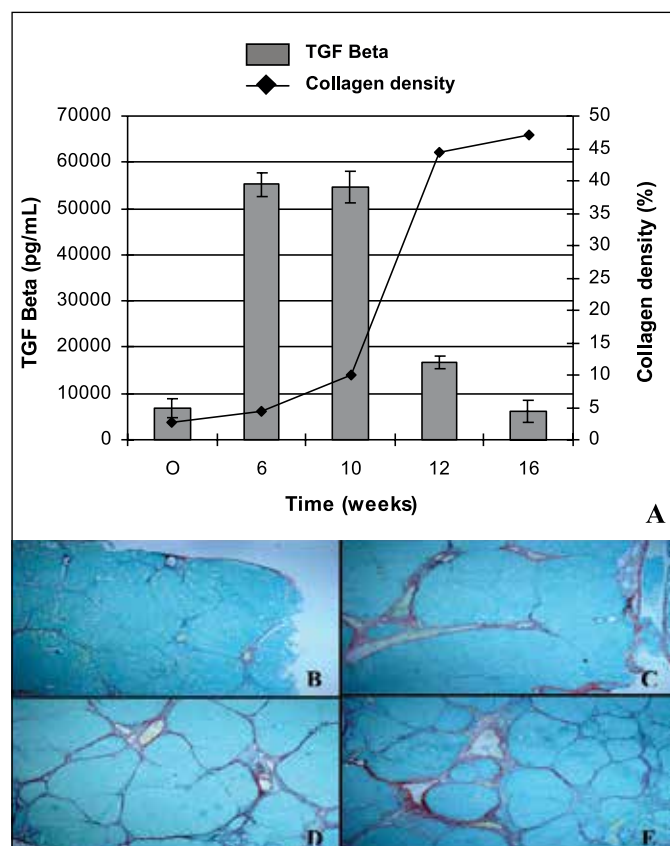


FIGURE 1. A) Mean levels of serum TGF $\beta 1$ (ng/mL) and collagen density (%) at different times of Carbon Tetrachloride exposition. B-E) Liver tissue from Carbon Tetrachloride exposed rats stained with Sirius Red. B) 6 weeks, C) 10 weeks, D) 12 weeks, E) 16 weeks. 200X

there was a significant reduction in TGF $\beta 1$ serum levels that at week 16 were similar to those of control animals. A moderate negative correlation was observed (-0.48 ; $P < 0.05$) between liver collagen content and TGF $\beta 1$ serum levels.

DISCUSSION

Chronic liver diseases represent a major public health problem, accounting for important morbidity and mortality worldwide. Despite their different etiologies, chronic liver diseases have in common the formation and accumulation of fibrosis leading to a progressive distortion of hepatic architecture. The management of these disorders depends, in many cases, on the amount and progression of liver fibrosis. Identifying the natural history of fibrosis deposition is important to evaluate the severity of liver damage and to establish the prognosis⁽¹²⁾.

In recent years, an increasing number of reliable non invasive methods became available to assess the development of liver fibrosis. Their utility is accepted in advanced fibrosis but an important limitation of these tests is the inability to detect mild stages of fibrosis. In humans, serum hyaluronic acid was the parameter that alone presented the best diagnostic accuracy in the assessment of hepatic fibrosis in chronic hepatitis C⁽¹³⁾ and serum levels of TGF $\beta 1$ correlated with the rate of fibrosis progression in hepatitis C adult patients⁽²⁾. In children with biliary atresia serum TGF $\beta 1$ was lower at the time of liver transplant compared with the time of diagnosis⁽¹⁵⁾.

Although TGF $\beta 1$ is increased in CCl₄-induced cirrhosis in rats, the kinetics of this cytokine in serum is not well known. Nakatsukasa et al.⁽¹¹⁾ studied the tissue expression of TGF $\beta 1$ in the liver of animals that received CCl₄ for 12 weeks and observed fluctuations along the time but without a defined tendency⁽³⁾. In the present work we show a marked decrease in serum TGF $\beta 1$ at end stage liver disease. At week 16, all animals in our study presented well established cirrhosis, with regenerative nodules and increased collagen density, which suggests that changes in vascular permeability may be occurring; similar to what has been observed in children with biliary atresia⁽¹⁵⁾. A similar study was conducted with serum laminin in the CCl₄ fibrosis model. Although a correlation between increased laminin and the degree of fibrosis has been observed, only cirrhotic animals showed values statistically higher than controls⁽¹⁶⁾.

Taken together, these results suggest that biomarkers are useful for the diagnosis or the exclusion of cirrhosis in animal models but that caution must be taken in order to interpret these results and correlate them with histological findings.

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RESUMO – Contexto - A citocina TGF β 1 (Fator Transformador de Crescimento, TGF β 1) desempenha um papel fundamental na fibrogênese, mas sua importância como biomarcador da doença hepática ainda tem sido debatida. **Objetivo** - Quantificar o TGF β 1 sérico e estudar a sua associação com o conteúdo de colágeno no tecido hepático em ratos expostos ao Tetracloreto de Carbono (CCl₄). **Métodos** - Ratos foram submetidos ao modelo de fibrose por CCl₄ e sacrificados após 6, 10, 12 e 16 semanas de tratamento. Os níveis séricos de TGF β 1 foram quantificados por ELISA e a densidade de colágeno foi definida por morfometria. **Resultados** - Os níveis séricos de TGF β 1 aumentaram entre 6 e 10 semanas, enquanto a densidade de colágeno aumentou entre 12 e 16 semanas. Foi detectada uma correlação negativa entre a deposição hepática de colágeno e a concentração sérica de TGF β 1 ($r = -0,48$; $P < 0,05$). **Conclusão** - O nível sérico de TGF β 1 foi inversamente proporcional à intensidade do colágeno no fígado de ratos com cirrose por CCl₄, o que indica que seu uso como biomarcador em estágios avançados da doença pode ter utilidade limitada.

DESCRITORES – Cirrose hepática experimental. Fator de Crescimento Transformador beta 1. Colágeno. Tetracloreto de Carbono. Ratos.

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