Partial hepatectomy and mediators of inflammation decrease the expression of liver α_2-HS glycoprotein gene in rats

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Liver mRNA levels of two acute phase reactant (APR) proteins, α_2-HS glycoprotein (a major negative APR) and α_1-acid glycoprotein (a major positive APR) were measured in male rats at different times after the administration of turpentine, of tumor necrosis factor, or following partial hepatectomy. In every case, a marked decrease in mRNA levels of α_2-HS glycoprotein was observed which reached a maximum at 24 h. A concomitant increase of α_1-acid glycoprotein mRNA levels was observed under the same conditions. These results indicate that the decreased levels of α_2-HS glycoprotein induced by the acute-phase response following inflammatory mediators and partial hepatectomy are due to a down-regulation of the gene expression of this protein in rat liver.

Acute phase; Liver protein synthesis; Cytokine; mRNA

1. INTRODUCTION

α_2-HS glycoprotein (AHSG) is a human plasma glycoprotein synthesized by the liver [1]. It has been shown to be involved in a number of functions such as opsonization [2], endocytosis [3], brain development [4], and formation of bone tissue [5]. Nevertheless, its precise biological role is still unknown. The human protein consists of two subunits, an A chain of 282 amino acids [6] and a B chain of 27 amino acids [7]. Interestingly, the corresponding cDNA sequence showed the A and B chains to be encoded by a single mRNA transcript [8].

Plasma concentrations of AHSG fall significantly following trauma [9] and during inflammatory response [10], and this protein is considered as the example of the so-called 'negative acute phase reactants'. In vitro we have shown that the synthesis of AHSG in liver cells was down-regulated by inflammatory mediators produced by activated monocytes, including IL-6 and IL-1 [11]. We have further shown that, in this in vitro system, the rate of synthesis of AHSG was closely correlated with the changes in its corresponding mRNA levels [11]. AHSG mRNA transcripts are found not only in human hepatoma cell lines, but also in rat liver [8], although the corresponding protein in the rat has not been completely characterized.

In the present study, we have examined the changes in the mRNA levels for AHSG in adult rat liver in response to in vivo acute inflammation, injection of tumor necrosis factor or partial hepatectomy, and compared them to those of α_1-acid glycoprotein (ORM), a typical 'positive' acute phase reactant.

2. MATERIALS AND METHODS

2.1. *Animals*

Male Sprague-Dawley adult rats (weight 180-200 g), purchased from Charles River (France), were maintained on a 12 h light/12 h dark cycle, with a standard laboratory chow, and water available ad libitum.

The effect of TNF was studied by injecting subcutaneously 20 μg of recombinant murine TNF (spec. act. 1.6×10^7 U/mg, kindly provided by Dr W. Fiers, Ghent, Belgium). Acute inflammation was induced by a single subcutaneous injection of 10 ml turpentine oil/kg body weight in the lumbar region. Partial hepatectomy was performed between 10.00 and 13.00 h according to the technique described by Higgins and Anderson [12], removing an average 75% of the liver. Liver mRNA analyses were performed after removal of the livers under ether anesthesia at different times following TNF injection, experimental inflammation, or partial hepatectomy.

2.2. *cDNA probes*

A plasmid containing an α_1-acid glycoprotein (ORM) cDNA insert was a generous gift of Dr Taylor (Gladstone Foundation Laboratory, San Francisco) and α_2-HS glycoprotein (AHSG) cDNA was obtained as described [13]. The two purified cDNA inserts (25-50 μg) were radiolabeled with [α-^{32}P]dCTP, using the random primer technique developed by Feinberg and Vogelstein [14].

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Abbreviations: AHSG, α_2-HS glycoprotein; ORM, α_1-acid glycoprotein; TNF, tumor necrosis factor

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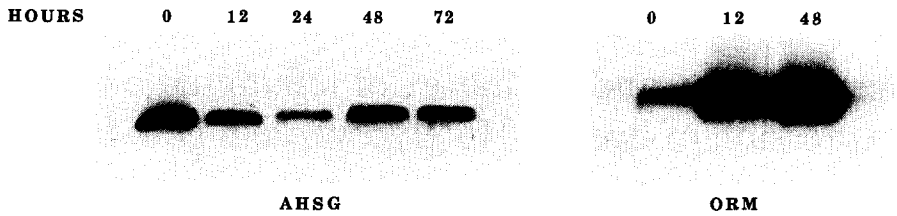

Fig. 1. Northern blot analysis of rat liver RNA extracted at 0, 12, 24, 48 and 72 h after *turpentine injection*. 50 μg aliquots of total liver RNA were separated on denaturing agarose gels. The RNAs were blotted onto nitrocellulose and hybridized with ^{32}P-labeled cDNA probes corresponding respectively to AHSG and ORM.

2.3. *RNA preparation*

Livers were homogenized in 4 M guanidium thiocyanate and RNAs were isolated by centrifugation through a 5.7 M CsCl layer as described [15]. RNA concentrations were determined by measuring the absorbance at 260 nm. The integrity of RNA preparations was controlled by agarose electrophoresis and visualization of the 18 S and 28 S ribosomal RNA bands after ethidium bromide staining.

2.4. *Northern blot analysis*

Samples of total RNA were denatured for 15 min at 55°C in 10 mM sodium phosphate buffer, pH 7.0 containing 2.2 M formaldehyde and 50% formamide, then applied to 1.2% agarose gels containing 2.2 M formaldehyde and separated by electrophoresis at 70 V for 4 h. Transfer was performed for at least 12 h onto Hybond nylon filters in 20× SSC [16]. The filters were baked and hybridized. Hybridization was performed at 42°C overnight and filters were washed under medium stringency conditions (6×SSC at room temperature for 4×5 min, then 2×15 min in 2× SSC, 1% SDS at 65°C). In some cases, after autoradiography, hybridization bands were quantified by densitometric scanning.

3. RESULTS

3.1. *Effect of experimental inflammation on the levels of α_2-HS glycoprotein and α_1-acid glycoprotein mRNAs*

We have compared the time course variations of AHSG mRNA in liver cells of adult male Sprague-Dawley rats after subcutaneous injection of turpentine. Fig. 1 shows that the decrease of the AHSG mRNA levels, as evidenced by Northern blotting, was obvious at 12 h and was maximum at 24 h. Thereafter, the levels of AHSG mRNA increased to come back to normal at 72 h. As a control, similar mRNA blots were hybridized with a cDNA probe for ORM, known as being a strong positive acute phase protein in man and in rats [17]. Results (Fig. 1) show that ORM mRNA levels were dramatically increased at 12 and 48 h following injection of turpentine.

3.2. *α_2-HS glycoprotein and α_1-acid glycoprotein mRNA changes after injection of tumor necrosis factor*

Levels of AHSG and ORM mRNA were determined in rat liver at various times following injection of TNF. As illustrated in Fig. 2, TNF injection caused a 2.2-fold reduction at 12 h and a 1.6-fold reduction at 24 h (as estimated by densitometric scanning) of the mRNA concentration for AHSG. In contrast, mRNA for ORM increased 6.1-fold at 12 h and 9-fold at 24 h following TNF injection.

3.3. *Effect of partial hepatectomy on the levels of α_2-HS glycoprotein and α_1-acid glycoprotein*

Levels of AHSG mRNA were determined in extracts of livers obtained from male adult rats at various times following hepatectomy. As evidenced by Northern blotting (Fig. 3), AHSG mRNA levels dropped substantially, being barely detectable 24 h after partial hepatectomy, and they rose progressively to attain normal

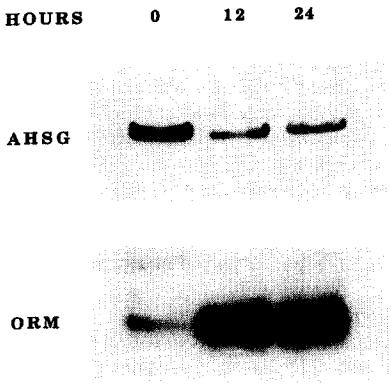

Fig. 2. Time course analysis of the effect of *TNF injection* (20 μg) on the expression of the AHSG and ORM genes. Northern blot analysis of 40 μg aliquots of total liver RNA extracted at 0, 12 and 24 h was performed as described in Fig. 1.

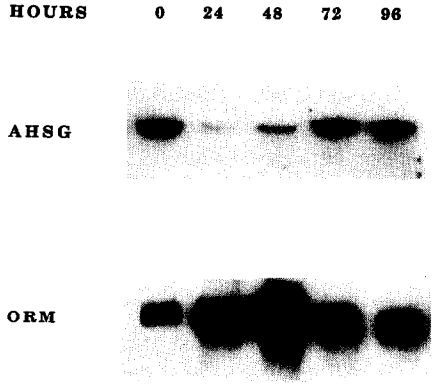

Fig. 3. Changes in the levels of AHSG and ORM mRNA in liver after *hepatectomy* analyzed by Northern blot of rat liver RNA extracted at 0, 24, 48, 72 and 96 h. 50 μg aliquots of total liver RNA were analyzed as described in Fig. 1.

values 72 h following hepatectomy. In contrast, levels of ORM mRNA were highest 48 h following hepatectomy and decreased progressively at 72 and 96 h.

4. DISCUSSION

In contrast to a number of human plasma proteins which are increased following infection, inflammation and malignancy, the plasma levels of AHSG are consistently decreased in trauma patients [9] or in patients with an inflammatory process due to severe bacterial infection [10]. Nevertheless, the precise reason for this was unknown, and, in particular, whether it reflected a decreased synthesis or an increased catabolism of the protein [10]. We have recently shown that inflammatory mediators from LPS-activated monocytes were able to decrease not only the synthesis of AHSG by liver cells in vitro, but also its mRNA levels [11]. In the present work, we have shown that, in vivo, rat liver AHSG mRNA levels were consistently decreased following three different stimuli known to induce the acute phase response, i.e. experimental inflammation, TNF injection and partial hepatectomy. Together with our previous results, this strongly favors the hypothesis that a decreased expression of the AHSG gene is responsible for the decreased values of this protein in plasma.

Liver AHSG mRNA levels were notably reduced 24 h after turpentine treatment, as shown recently for albumin [18]. Turpentine is known to produce a typical acute phase response through monocyte activation, and, among the multiple cytokines which are released by activated monocytes, IL-6 stimulates the synthesis of the full spectrum of the positive acute phase proteins in human liver cells [19,20]. Nevertheless, other cytokines may also be important, and, in particular, in the present study, TNF was found to reduce markedly AHSG mRNA levels. Similarly, TNF-α has been shown to be more efficient in vitro than IL-6 in decreasing the synthesis of two other negative acute phase proteins, transferrin and albumin [20]. Whether this decrease reflects a direct response to TNF, or an indirect effect through the liberation or amplification of IL-1 and IL-6 release, or a combination of both remains to be determined. In this respect, the decrease in hepatic mRNA levels of AHSG after turpentine injection could also be mediated, at least in part, by TNF produced by stimulated monocytes. Indeed, TNF secretion precedes the production of IL-1β and IL-6, and is a necessary stimulus for their release in vivo during acute infection in baboons [21].

Finally, partial hepatectomy induced at 24 h a decrease in AHSG mRNA levels comparable to, if not stronger than that observed following turpentine or TNF injection. This result is in agreement with the study of Fung et al. [22], who showed a decrease in the transcription of the transthyretin (a negative acute phase protein) gene following partial hepatectomy in rats.

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