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Increased gene expression of liver SREBP-2 in experimental chronic renal failure

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Abstract

Sterol regulatory element-binding protein-2 (SREBP-2) is a transcription factor regarded as the main regulator of cholesterol homeostasis. Therefore, increased level of SREBP-2 could be responsible for hypercholesterolemia, which is observed in experimental chronic renal failure (CRF). This study was designed primary to evaluate the impact of experimental CRF (5/6 nephrectomy model) on rat liver SREBP-2 gene expression. In CRF rats, a twofold increase in SREBP-2 mRNA level, as well as in mature SREBP-2 protein abundance was found, when compared to control animals. It was associated with enhanced activity and mRNA abundance of liver HMG-CoA reductase, a rate-limiting enzyme for cholesterol biosynthesis. A twofold increase in liver cholesterologenesis rate was also noted. We conclude that experimental CRF is associated with increased liver SREBP-2 gene expression. This is probably the cause for enhanced HMG-CoA reductase gene expression and, consequently, for increase in liver cholesterol synthesis in CRF rats. Despite increased SREBP-2 gene expression we found LDL-receptor mRNA level to be lower than in controls, suggesting SREBP-2 independent mechanisms of LDL-receptor transcriptional regulation in CRF rats. Enhanced cholesterol synthesis and decreased LDL-receptor mRNA level are probably responsible for an almost fourfold increase in serum cholesterol concentration in CRF rats.

Keywords: Chronic renal failure; Sterol regulatory element-binding protein-2 (SREBP-2); HMG-CoA reductase; LDL-receptor; Hypercholesterolemia; Cholesterol synthesis

1. Introduction

Cardiovascular and cerebrovascular diseases are the main causes of increased morbidity and mortality in patients suffering from chronic renal failure (CRF), compared to general population [1]. This is mainly due to exacerbation of atherosclerotic processes in these patients. Lipid disturbances play a major role in atherosclerosis development. Therefore, they are considered as risk factors for vascular related complications. Hypercholesterolemia is one of the main lipid disorders, being a constant feature of experimental CRF. In our previous studies, we showed that, at least in part, it is caused by enhanced cholesterol biosynthesis in CRF rats [2–4]. Moreover, we showed that gene expression of HMG-CoA reductase, the rate-limiting enzyme for cholesterologenesis, was increased in livers of CRF animals [2,4].

Regulation of cholesterol biosynthesis occurs via a family of transcription factors designated as sterol regulatory element-binding proteins (SREBPs) [5]. There are three known members of SREBP family: SREBP-1a, SREBP-1c and SREBP-2, of which the last one is regarded as the main regulator of cholesterologenesis [6–9]. Thus, SREBP-2 may represent a suitable candidate for regulation of cholesterol synthesis in diseases associated with hypercholesterolemia.

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SREBP genes encode membrane proteins that are bound to endoplasmic reticulum (ER) membranes and serve as precursors for active transcription factors, called mature or nuclear SREBPs. Transcription factor release is controlled by SREBP cleavage-activating protein (SCAP), which escorts SREBP precursor from ER to the Golgi apparatus. When cellular cholesterol level is low the *C*-terminal regulatory domain of SREBP precursor interacts with SCAP and, in consequence, the molecule is transported to Golgi apparatus where SREBP precursor is clipped by site-1-protease. Then, site-2-protease clips the *N*-terminal SREBP intermediate releasing the mature transcription factor. Upon translocation to the nucleus it binds to SRE sequences within the promoters of target genes and, in consequence, enhances their transcription. The opposite situation occurs in case of sterol overload. SREBP remains bound to endoplasmic reticulum membranes, expression of target genes ceases and cellular cholesterol level decreases [5–7].

Recently, we have shown higher level of SREBP-1 in white adipose tissue of CRF rats, which is strictly associated with higher rate of fatty acid synthesis in adipose tissue [10]. Levi and co-workers [11,12] found increased renal expression of SREBP-1 and SREBP-2 associated with increased renal accumulation of triglycerides and cholesterol, as well as with glomerulosclerosis and proteinuria. Since: (a) liver is the main organ synthesizing cholesterol (and other lipids); (b) cholesterol synthesis is increased in livers of CRF rats [2–4] and (c) SREBP-2 is a transcription factor controlling expression of the key genes of cholesterologenesis [6], the present work was designed to determine whether experimental CRF was associated with an increase in mRNA level and protein abundance of liver SREBP-2. In livers of the same animals, we also evaluated changes in: (a) HMG-CoA reductase mRNA level; (b) HMG-CoA reductase activity; (c) in vivo cholesterol biosynthesis and (d) LDL-receptor mRNA abundance.

2. Materials and methods

2.1. Induction of chronic renal failure

All experiments were performed under a protocol approved by the Ethical Committee of Medical University of Gdansk (4/2000). Male Wistar rats (8–9 week old, weight 230–250 g at the beginning of the study) were used. Animals were kept in individual cages, on a controlled lighting schedule, in which the room was illuminated from 7 a.m. to 7 p.m. daily. Experimental CRF was induced by our own modification of 5/6 nephrectomy model [13]. A standard diet for laboratory animals, "Labofeed H"-Poland was used [14].

2.2. Cholesterol biosynthesis in vivo

Experiment was carried out from 10 p.m. to 2 a.m., i.e. during maximal cholesterologenesis activity. A 10 mCi of tritiated water (0.2 ml) was administered intraperitoneally. After 1 h animals were killed under light ether anesthesia. Livers and serum were collected and frozen at −80 °C. After having collected material from all rats, livers were weighed and 2 g slices were used for analysis. They were placed into 10 ml of 15% solution of KOH in methanol with known amount of [^{14}C] cholesterol (about 4000 dpm), which was added as a check for recoveries. Mixtures were saponified at 65 °C for 3 h. The non-saponifiable lipids were extracted with *n*-hexane. The extracts were evaporated and digitonides were prepared according to Sperry and Webb [15]. Digitonides were dissolved in hot glacial acetic acid and aliquots were taken for both radioactivity and sterols determinations. Sterols were assayed with Lieberman–Burchardt reagent. Radioactivity was determined in Beckman liquid scintillation counter and corrected for quenching by external standard channels-ratio method.

Serum cholesterol, creatinine and urea concentrations were determined using Hitachi 704 autoanalyser.

2.3. Assay of HMG-CoA reductase activity

Freshly excised livers were rinsed three times with 0.9% NaCl and three times in sucrose medium containing: 0.3 M sucrose, 10 mM mercaptoethanol and 15 mM Tris–HCl (pH 7.4) buffer. Tissues were drained on filter paper and homogenized manually with buffered solution in a glass Potter–Elvehjem homogenizer. Homogenate was centrifuged twice at 15,000 × *g* for 10 min and the resulting supernatant was centrifuged at 105,000 × *g* for 60 min. Microsomal pellet was resuspended in imidazole buffer containing: 1 mM EDTA, 250 mM NaCl, 5 mM DTT and 50 mM imidazole–HCl (pH 7.4) buffer. HMG-CoA reductase activity was assayed by the method of Shapiro et al. [16].

2.4. Isolation of total RNA and RT PCR

Total cellular RNA was extracted from frozen livers by guanidinium isothiocyanate–phenol/chloroform method and the procedure was performed as described previously [17]. RNA concentration was determined from the absorbance at 260 nm and all samples had 260/280 nm absorbance ratios of about 2.0. First strand cDNA was synthesized from 1 μg of total RNA. Prior to amplification of cDNA, each RNA sample was treated with RNase-free DNase I (Fermentas, Lithuania) at 37 °C for 30 min. The cDNA was used as a template in Multiplex PCR reaction with housekeeping β-actin gene as an internal control [18,19]. The cDNA samples were amplified for 30 cycles at a final volume of 20 μl containing 1× PCR buffer, 3.5 mM $MgCl_2$, 0.5 mM dNTP Mix, 0.5 mM of sense and antisense primers and 0.5 U *Taq* DNA Polymerase (Fermentas, Lithuania). The conditions of all reactions were experimentally checked to ensure that the signals were in the linear range of the assay. Specific sense and antisense primers used for preparations of respective cDNA were: for HMG-CoA reductase 5′-ACATGATTTCCAAGGGTACGG-3′ and