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Retinoid metabolism during development of liver cirrhosis

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Abstract

The changes in retinoid metabolism have been documented in liver cirrhosis. However, the dynamic alterations in levels of this vitamin between circulation and liver during development of the liver cirrhosis are not well understood. The aim of this study was to measure retinoids in the liver and circulation in parallel, during and after development of cirrhosis induced by carbon tetrachloride and thioacetamide. Retinoid levels were measured by HPLC. A decrease in retinaldehyde and total retinol, together with an increase in retinoic acid was evident in liver from both carbon tetrachloride or thioacetamide treated rats within a month after initiation of treatment. Activity of enzymes involved in retinoid metabolism such as retinaldehyde oxidase, retinaldehyde dehydrogenase, and retinaldehyde reductase were decreased in the liver. In parallel, levels of retinol and retinaldehyde in the serum were increased while retinoic acid was decreased. This study indicates that during development of cirrhosis, there is reciprocal transfer of retinoid metabolites between the circulation and the liver.
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Keywords: Retinol; Retinoic acid; Retinaldehyde; Metabolizing enzymes

Retinoids (vitamin A and its derivatives) have profound effect on morphogenesis and are essential for vision, reproduction, growth, differentiation, and maintenance of the health of organisms [1,2]. Retinoid homeostasis is maintained by a number of interconnected systems, of which the liver is a critical player, involved in homeostasis, metabolism, and storage of retinoids [3]. Vitamin A is absorbed from the diet in the intestine as α-carotene, β-carotene, and β-cryotoxanthin. These carotinoids are cleaved by the carotene cleavage enzyme to retinaldehyde, which is then metabolized to either retinol or retinoic acid by retinaldehyde reductase or retinaldehyde dehydrogenase, respectively. The retinol formed in the enterocytes is esterified with fatty acids by lecithin: retinol acyl transferase to form retinyl esters which are transported as chylomicron retinyl esters through circulation, which is then cleared by the liver [4,5]. In addition to the liver, 25–30% of chylomicron retinoids are cleared by extra hepatic tissues [5]. From the liver, retinol is transported to other tissues as a retinol–retinol binding protein (RBP)[1] complex, which forms protein–protein interactions with transthyretin (TTR) [6]. In addition, the circulation also contains all-*trans*-retinoic acid bound to albumin and soluble retinoyl-β-glucoronides.

In liver cells, retinoic acid taken from the circulation is bound to cellular retinoic acid binding protein (CRABP) I

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[1] *Abbreviations used:* RBP, retinol binding protein; TTR, transthyretin; BSA, bovine serum albumin; NAD, nicotinamide adenine dinucleotide; CCl_4, carbon tetra chloride; TAA, thioacetamide; HPLC, high performance liquid chromatography; TGF-β, transforming growth factor-β; RAREs, retinoic acid response elements; CRABP, cellular retinoic acid binding proteins.

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or II for transcriptional activation of vitamin A-responsive genes [4]. Alterations in retinoic acid metabolism have been noted in cirrhosis, [2,7,8] and decreased levels of retinol, RBPs, TTR, and β-carotene in the serum in patients with liver cirrhosis has been demonstrated [9–11]. We hypothesized that liver cirrhosis would result in a significant alteration in retinoid metabolism during the course of development of disease; with early changes progressively leading to profound alterations by the time cirrhosis is evident histologically. To understand these processes, we have followed retinoid levels in the liver during development of liver cirrhosis using two different animal models, in an effort to understand mechanisms involved in these changes during the disease.

Materials and methods

Tris (hydroxymethyl) aminomethane (Tris), *N*-[2-hydroxyethyl] piperazine-*n′*-[2-ethanesulfonic acid] (Hepes), bovine serum albumin (BSA), nicotinamide adenine dinucleotide (NAD), its reduced form (NADH), all-*trans*-retinol, all-*trans*-retinaldehyde, all-*trans*-retinoic acid, sodium pyruvate, α-ketoglutarate, L-alanine, aspartic acid, *para*-nitrophenol, *para*-nitrophenyl phosphate, and 4-hydroxy proline were obtained from Sigma Chemical (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Animals

Adult Wistar rats of both sexes (125–150 g), exposed to a daily 12 h light–dark cycle and fed water and rat chow ad libitum were used for this study. Four groups of animals were used (Control, phenobarbitone control, TAA, and CCl_4 treatment). Each group comprised of six animals. This study was approved by Institutional Animal Ethics Committee (IAEC).

Induction of liver cirrhosis in rats

Cirrhosis was induced by administering carbon tetra chloride (CCl_4) or thioacetamide (TAA). For CCl_4-induced liver cirrhosis, rats were treated with phenobarbitone (35 mg/dl) in tap water, which was the source of drinking water for 14 days before initiation of the experiment. Intragastric instillation of CCl_4 in coconut oil was given using a 2.5 ml syringe attached to 2 mm-diameter tygon tubing twice a week under light halothane anesthesia. The initial dose of CCl_4 was 40 μl/rat. Subsequent doses were adjusted based on the change in body weight. Control animals received phenobarbitone, along with coconut oil alone without CCl_4 [12]. Treatment of phenobarbitone and CCl_4 was stopped after each time point during the experiment, namely 1, 2, 3, 4, and 5 months and the animals were sacrificed by decapitation after 10 days. For thioacetamide-induced liver cirrhosis, rats were administered intraperitoneal injection of TAA (200 mg/kg, ip) in saline twice a week for 1, 2, 3, 4, and 5 months. Control rats received vehicle alone [13]. After each treatment period, TAA was stopped and the animals were sacrificed after one week. Fully developed micro- and macro-nodular cirrhosis was confirmed by histology and circulating biochemical parameters. Blood was collected by direct heart puncture and serum separated. Care was taken to protect the tissue and serum from light exposure for retinoid estimations.

Histology

Liver tissue was fixed in 10% buffered formalin and processed. Four-micron sections were cut and stained with hematoxylin and eosin and observed under light microscope.

Preparation of liver homogenate

The liver was washed thoroughly of blood, minced and homogenized with 8 volumes of homogenization buffer containing 230 mM mannitol, 70 mM sucrose, and 3 mM Hepes, pH 7.4, using a Porter-Elvehjem homogenizer and used for the measurement of retinoids [14].

Retinol, retinaldehyde, and retinoic acid extraction and quantitation by high performance liquid chromatography

The liver homogenate corresponding to approximately 1 mg protein was mixed with an equal volume of 100% ethanol and 0.025× volume of 0.1 N HCl. Neutral and acidic retinoids were extracted twice with 3× volume of hexane. Extracted fractions were dried under nitrogen and reconstituted in 100% ethanol for high performance liquid chromatography (HPLC) separation. Retinoids were separated on a Shim-pack CLC-SIL silica column running at 1 ml/min using the mobile phase (hexane/dioxane/acetic acid, 92:8:0.1). Retinol, retinaldehyde, and retinoic acid were monitored at 350 nm [15]. Quantitation was performed by relating the area of the peak to areas obtained by the analysis of known quantities of retinoid standards and expressed as picomoles per milligram protein. For serum retinoids, 0.5 ml serum was mixed with equal volume of ethanol and 0.025 ml of 0.6 N HCl and extracted as mentioned above and expressed as picomoles per milliliter of serum. The recovery of these retinoids by this method was around 85%.

Saponification of retinyl esters

For saponification of retinyl esters to retinol, the liver homogenate corresponding to 0.1 mg protein was mixed with an equal volume of 5% ethanolic potassium hydroxide and 1 M sodium chloride. The mixture was incubated at 65 °C for 2 h in dark [5]. The reaction was stopped by adding equal volume of 100% ethanol and extracted as

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