Biochemical and Molecular Actions of Nutrients

Dietary Nucleotides Enhance the Liver Redox State and Protein Synthesis in Cirrhotic Rats

María José Pérez,* Fermín Sánchez-Medina, Maribel Torres, † Angel Gil, and Antonio Suárez†

Department of Biochemistry and Molecular Biology, University of Granada, Granada, Spain; *Department of Physiology and Pharmacology, University of Salamanca, Salamanca, Spain; and †Department of Experimental Biology, University of Jaen, Spain

ABSTRACT Cirrhosis is characterized by altered lipid and protein metabolism and an excessive accumulation of extracellular matrix components. The aim of this work was to determine the effect of dietary nucleotide intake on the intracellular pools of nucleic acids and nucleotides, hepatic redox state, and protein synthesis during cirrhosis. Rats were given 300 mg/L thioacetamide (TAA) in drinking water and were fed diets without (TAA+Nt) or with nucleotides (Nt) (TAA+Nt, 3 g each of AMP, inosine 5′-monophosphate, CMP, GMP, and UMP per kg diet) for 4 mo. The degree of liver histological injury was less in group TAA+Nt than in TAA+Nt. The intake of nucleotides significantly increased the hepatic concentration of total nucleotides, adenine nucleotides, and ATP+ADP+AMP. Interestingly, the concentration of CDP-choline, a nucleotide necessary for phospholipid synthesis, was significantly higher in TAA+Nt than in TAA+Nt. The hepatic pyruvate:lactate (P = 0.075) and acetoacetate:β-hydroxybutyrate (P < 0.05) ratios, indicators of cytosolic and mitochondrial redox states, were lower in TAA+Nt than in TAA+Nt. The total protein concentration was higher in the livers of TAA+Nt than in TAA+Nt. Although there were no differences in the expression of the albumin gene, the hepatic albumin concentration was significantly higher in TAA+Nt than in TAA+Nt. These data indicate that the reduction of liver injury in nucleotide-supplemented rats may be due to the increased intracellular availability of key metabolic nucleotides, the restoration of mitochondrial function, and the augmentation of protein synthesis. J. Nutr. 134: 2504–2508, 2004.

KEY WORDS: • dietary nucleotides • thioacetamide • cirrhosis • redox state • protein synthesis

Liver cirrhosis is associated with important metabolic changes, including altered amino acid, carbohydrate, lipid, vitamin, and mineral metabolism. The inability to adequately use glucose and fat as tissue fuels increases protein catabolism, which is the limiting factor in satisfying the nutritional requirements of patients with cirrhosis. Adequate nutritional support is essential for the regeneration of damaged hepatocytes in liver cirrhosis and contributes to decreased morbidity of the disease in humans (1).

Nucleotides are conditionally essential nutrients that modulate lipid metabolism, immune function, and intestinal microbiota and have a reparative effect in pathological conditions that demand intense nucleic acid and protein synthesis, such as intensive growth and repair of certain tissues (2). Our research group studied the reparative effects of dietary nucleotides in rats with experimental liver cirrhosis induced by thioacetamide (TAA),3 a model that has a number of metabolic alterations similar to those found in the human disease. Histological analysis of liver sections in TAA-induced cirrhotic rats showed increased hepatocyte binuclearity and reduced extension of liver damage in rats fed nucleotides (3). Dietary supplementation with nucleotides normalized protein concentration and serum amino acids as well as linoleic and arachidonic acid concentrations in liver microsomes (4). We recently demonstrated that reduced hepatic fibrosis in rats fed a nucleotide-supplemented diet is due to significantly lower prolyl-4-hydroxylase activity, reduced hepatic protein concentration and TIMP-1 gene expression, and markedly increased total collagenase activity (5).

These results suggest that exogenous nucleotides play an important role in the repair and regeneration of cirrhotic liver. Because deprivation of nucleotides significantly reduces the hepatic protein synthesis rate (6), a mechanism that may be responsible for tissue improvement is that incorporation of exogenous nucleotides into intracellular nucleotides pools would increase nucleic acid and protein synthesis, promoting cell growth and proliferation under pathological conditions. However, to date no data concerning the effect of exogenous nucleotides on the concentration of total nucleotides and nucleic acids and on protein synthesis in liver cirrhosis have been reported. To address these questions, we determined the nucleotide concentration of liver, hepatic redox state, and

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2 To whom correspondence should be addressed. E-mail: asuarez@ugr.es.

3 Abbreviations used: ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; ER, rough endoplasmic reticulum; G-6-Pase, glucose-6-phosphatase; IMP, inosine 5′-monophosphate; R, healthy reference group; TAA, thioacetamide. TAA+Nt, cirrhotic rats fed semipurified diet; TAA+Nt, cirrhotic rats fed semipurified diet supplemented with 0.3 g each of AMP, IMP, CMP, GMP, and UMP per kg diet.

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hepatic albumin mRNA and protein concentration in TAA-induced cirrhotic rats fed nucleotides.

**MATERIALS AND METHODS**

**Materials.** Female Wistar rats, with an initial weight of 150–170 g, were supplied by the Animal Service of the University of Granada. Semipurified diets were supplied by Abbott Laboratories, and nonpurified standard rat diet was purchased from Panlab (Diet No. A04). Both diets were stored at 4°C under nitrogen.

**Experimental design.** All rats were treated in accordance with the recommendations of the American Physiological Society (Council of Europe, 1982). Adult female Wistar rats were housed in wire-bottom cages with a 12-h light/dark cycle. The rats were divided into 3 groups of 10 rats each and were fed for 4 mo: 2 of these groups were deprived of food for 12 h, anesthetized with a 250 g/L solution of urethane at a dose of 1 mL/100 g body wt, and killed by terminal bleeding. The livers were removed and a slice of the right lobule was fixed in formaldehyde solution and embedded in paraffin. Liver histology.

**Enzyme activities.** Enzyme activities were determined in serum or crude homogenates and units were expressed per milligram of protein in the supernatant. Aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were assayed in serum and liver by the methods described in Bergmeyer’s handbook (8). Liver glucose-6-phosphatase was determined by measuring the phosphorus released in the presence of glucose 6-phosphate (9). The protein concentration was determined by the Bradford method (10).

**Cellular redox state.** Liver redox state was determined in freeze-clamped samples by the calculation of acetoacetate:hydroxybutyrate and pyruvate:lactate ratios. Liver acetoacetate and β-hydroxybutyrate concentrations (11) and the lactate concentration (12) were determined as described previously. Pyruvate concentration was determined using a diagnostic kit purchased from Sigma-Aldrich S.A. (Ref. No. 726).

**Acid-soluble nucleotides.** Total free nucleotides were determined in freeze-clamped samples by ion-exchange HPLC (13).

**Liver total DNA and RNA concentrations.** Liver DNA was measured on liver homogenates using a fluorimetric assay (14), and liver RNA was measured by the method of Fleck and Munro (15).

**Northern blot analysis.** Total RNA was extracted from 100 mg of fresh rat liver tissue as described previously (16). Ten micrograms of denatured total RNA was electrophoresed, transferred to nylon filters, and fixed with a UV crosslinker. The filters were sequentially hybridized with an albumin (α-32P)dCTP-labeled cDNA probe, with an 18S rRNA (α-32P)dCTP-labeled oligonucleotide (5′-CAT GGT AGG CAC GGC GAC TAC CAT-3′), and with a (α-32P)dCTP-labeled rat glyceraldehyde-3-phosphate dehydrogenase cDNA probe to validate Northern blot results.

**Liver albumin.** Liver concentration of albumin was quantified by an indirect sandwich ELISA as previously described (5), using an anti-rat albumin sheep antibody and purified albumin as a standard. Western blot analysis was used to confirm the results obtained by ELISA (17). Rat albumin and equal amounts of total liver protein per sample were detected using the same anti-rat albumin antibody and the chemiluminescence light detection kit from Amersham-Pharmacia.

**Statistical analysis.** Results are means ± SEM, n = 10. An unpaired Student’s t test was used to compare the TAA-treated TAA−Nt and TAA+Nt groups. Differences with P-values < 0.05 were considered significant. All data were analyzed using BMDP software, PC 90 version (BMDP Statistical Software).

**FIGURE 1** Electron micrographs of liver in cirrhotic rats fed diets with (TAA−Nt, A and B) or without (TAA−Nt, C and D) nucleotides. N, nucleus; Nu, nucleolus; M, mitochondria; R, endoplasmic reticulum; L, lipid droplets. Original magnification X2200. Scale bar = 1 μm.
RESULTS

**Histology.** Chronic intoxication with orally administered TAA affected the ultrastructure of hepatocytes in the TAA-Nt group. The cytoplasm contained few and small mitochondria and they appeared to be electron-dense (Fig. 1A). Numerous lipid droplets were seen in the cytoplasm. Nuclei became edematous and increased in size until they occupied a large proportion of cell volume and the nuclear envelope was electron-dense (Fig. 1B). The nucleoli were enlarged, granular, and vacuolated. The rough endoplasmic reticulum (ER) became disaggregated and many of its cisternae were dispersed, dislocated, and altered in length.

In contrast, the TAA+Nt had less histological injury than TAA−Nt. Normal-appearing mitochondria and large amounts of ER were observed in the hepatocyte cytoplasm in TAA+Nt (Fig. 1C). The shape and size of the nucleus and nucleoli were normal in TAA+Nt (Fig. 1D). Lipid droplets were substantially smaller in TAA+Nt than in TAA−Nt.

**Biochemical analyses.** Liver ALAT and ASAT activities and serum ASAT activity did not differ between the groups. Liver G-6-Pase activity was higher and serum ALAT activity was lower in TAA+Nt than in TAA−Nt (P < 0.01, Table 1).

The hepatic acetoacetate:β-hydroxybutyrate ratio was higher (P < 0.05, Fig. 2A) and the pyruvate:acetocetate ratio tended to be higher (P = 0.075, Table 1) in TAA+Nt than in TAA−NT.

The liver protein concentration was higher in TAA+Nt than in TAA−Nt (P < 0.05, Table 1). Hepatic DNA and RNA and 18S RNA concentrations did not differ between the groups (Table 1). Except for UDP-glucose levels (Table 1), the concentrations of total nucleotides (Fig. 2B), adenine nucleotides, CDP-choline, and the sum of ATP+ADP+AMP were higher in TAA+Nt than in TAA−Nt (P < 0.05, Table 1).

TAA+Nt had a greater hepatic albumin concentration (P < 0.01 Table 1; Western blot in Fig. 2C) although the level of albumin mRNA did not differ between the groups (Table 1).

DISCUSSION

Our group and others extensively studied the beneficial effects of nucleotides in the repair and restoration of the structure and functioning of liver after injury or partial hepatectomy (4,5,18–20). The mechanism by which exogenous nucleotides exert their beneficial effect has yet to be established. In this paper, we present evidence suggesting that exogenous nucleotides are incorporated into intracellular pools and enhance hepatocyte functionality and protein synthesis.

Oral ingestion of TAA generates experimental cirrhosis in rats, a model that resembles human cirrhosis because both share a number of metabolic and histological alterations (4,5). Transmission electron microscopic analysis of liver sections

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<td><strong>Serum and liver biochemistry of healthy (R) and orally thioacetamide-treated rats fed diets with (TAA+Nt) or without (TAA−Nt) nucleotides</strong></td>
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<sup>1</sup> Values are means ± SEM, n = 10. Different letters indicate differences between groups, P < 0.05.
<sup>2</sup> AU, arbitrary units; G-6-Pase, glucose-6-phosphatase.
DIETARY NUCLEOTIDES AND CIRRHOSIS IN RATS

2507

FIGURE 2  Hepatic acetoacetate:β-hydroxybutyrate ratio (A), total nucleotide concentration (B), and albumin protein (C) in healthy (R) and cirrhotic rats fed diets with (TAA+Nt) or without (TAA−Nt) nucleotides. Values are means ± SEM, n = 10. Different letters indicate differences between groups, P < 0.05.

(Fig. 1) showed group TAA−Nt hepatocytes with edematous nuclei, vacuolated nucleoli, disaggregated ER, and cytoplasm containing lipid droplets and few mitochondria compared with group TAA+Nt hepatocytes. Serum and liver ALAT and ASAT activities confirmed the establishment of a TAA-induced cirrhotic process in rats (Table 1).

Because alterations in nuclear size and ER aggregation could be related to changes in nucleic acid, total nucleotide concentration, and ER integrity, we measured total liver DNA, RNA, 18S rRNA, total nucleotide, ATP+ADP+AMP, adenine nucleotide, UDP-glucose and CDP-choline values, and G-6-Pase activity. Our group recently reported that exogenous nucleotides are actively incorporated into intracellular pools (21) and enhance DNA synthesis and proliferation of fetal hepatocytes (22). Under our experimental conditions, we did not detect significant differences in DNA, 18S rRNA, and RNA concentrations in livers of rats fed diets with or without nucleotides. However, the concentration of total nucleotides was substantially higher in TAA+Nt than in TAA−Nt (Fig. 2A). These results agree with those of Palombo et al. (20), who reported that dietary nucleotides contributed to maintain the hepatocyte concentrations of ATP in cold ischemic rats. Furthermore, hepatic G-6-Pase activity, a marker of ER integrity, was significantly higher in TAA+Nt than in TAA−Nt. These data indicate that the intake of nucleotides in the diet (1.5 g/kg diet) is enough to restore intracellular levels of free nucleotides and to enhance ER integrity in hepatocytes of cirrhotic rats.

Hepatic CDP-choline concentrations were higher in TAA+Nt than in TAA−Nt (P < 0.01, Table 1). Arnaud et al. (21) showed that exogenous nucleotides increased CDP-choline concentration in cultured fetal hepatocytes. CDP-choline is a nucleotide necessary for phospholipid synthesis and lipoprotein assembly. The elevation in CDP-choline levels by nucleotide dietary supplementation may explain the absence of intracellular lipid droplets (Fig. 1).

The mitochondria in hepatocytes of cirrhotic rats were altered and their number was reduced. To study the impact of nucleotide intake on mitochondrial function, we measured pyruvate:lactate (Table 1) and acetoacetate:hydroxybutyrate (Fig. 2B) ratios as indicators of cytosolic and mitochondrial redox states. Both ratios were lower in TAA−Nt than in TAA+Nt, an indication of an altered electron transport chain. The concentrations of ATP+ADP+AMP and adenosine nucleotides (Table 1), which are the carriers of electrons and the final acceptors of the energy generated during the mitochondrial energy coupling process, were lower in TAA−Nt than in TAA+Nt. Hernández-Muñoz et al. (23) obtained similar results in rats with carbon tetrachloride−induced cirrhosis and found a correlation between cellular redox state and collagen metabolism. These biochemical alterations in hepatocytes of TAA−NT rats may cause uncoupling of the electron transport chain and oxidative phosphorylation, which would result in NADH and lactate accumulation and in an insufficient energy synthesis rate. We hypothesize that incorporation of preformed nucleotides enhances the efficacy of oxidative phosphorylation, electron transport, and the turnover between oxidized and reduced forms of NAD, which would stimulate the production and storage of energy in hepatocytes.

Most of the energy generated by a cell is used to drive protein synthesis. Our group previously reported that deprivation of dietary nucleotides produced significant reductions in hepatic protein synthesis (6). To determine the effect of dietary nucleotides on liver protein synthesis during cirrhosis, we determined the hepatic protein concentration and used albumin as a marker of hepatic protein synthesis because it is one of the major proteins produced and secreted by the liver. Hepatic total protein and albumin concentrations were significantly higher in rats fed nucleotides. Since the expression of the albumin gene did not differ between the groups, these data suggest that dietary nucleotide intake by cirrhotic rats enhances the efficiency of protein synthesis in the liver.

This is the first report that dietary nucleotides increase the intracellular pool of nucleotides and improve the redox state and the efficiency of protein synthesis in cirrhotic rats. Based on these results, we conclude that the intake of nucleotides can improve liver function and reduce the extent of cirrhosis in rats. We contend that these results justify a study on the efficacy of nucleotide-supplemented formula in cirrhotic patients.
LITERATURE CITED


