CINC Y CIRROSIS HEPATICA: EVALUACION BIOQUIMICA E HISTOPATOLOGICA

Se indujo una cirrosis hepática experimental mediante la administración de tiocetamida. los animales cirróticos fueron divididos en dos grupos, un grupo recibió sulfato de cinc y el otro actúo como control. Los animales tratados con cinc mostraron una restauración de los niveles plasmáticos y hepáticos de cinc y cobre. De la misma forma, los niveles plasmáticos de aspartato aminotransferasa (ASAT), alanino aminitransferasa (ALAT), gamma-glutamil aminotransferasa (GGT) y bilirrubina total, descendieron significativamente. Los estudios al microscopio óptico mostraron una apariencia normal en la mayoría de los hepatocitos del grupo tratado con cinc en comparación a los animales cirróticos no tratados. Las cantidades de fibrina, reticulina y colageno, elevadas en los hígados cirróticos, disminuyeron tras el tratamiento con cinc. La tinción con PAS mostró la capacidad de los hepatocitos para almacenar glucógeno tras el tratamiento con cinc. Estos resultados revelan que el cinc podría tener algún efecto beneficioso en el tratamiento de la cirrosis hepática.

POTENCIAL DEL MONOGLICERIDO Y TRIGLICERIDO DEL DL-3-HIDROXIBUTIRATO EN NUTRICION PARENTERAL: SINTESIS Y ESTUDIOS BIOLOGICOS EN LA RATA

Los ésteres de cadena corta de los ácidos orgánicos se han mostrado prometedores como potenciales nutrientes en alimentación parenteral. La mayoría de glicéridos son insolubles en agua pero los de los cuerpos cetónicos muestran cierta solubilidad. Es interesante el hecho de que el triglicérido del 3-hidroxibutirato es soluble en agua mientras que el triglicérido del acetoacetato no lo es. Los mono y triglicéridos del DL-3-hidroxibutirato fueron sintetizados y ensayados para evaluar su toxicidad y su valor nutricional como nutrientes parenterales. Ambos compuestos tienen una densidad calórica estimada de 19.7 Kj/g (4.7 Kcal/g) y son hidrosolubles. Los compuestos fueron infundidos en ratas durante 7 días a un ritmo que proporcionaba 113 Kj/día y además, se administro una dieta oral hipocalórica. Un grupo control de animales recibió la misma dieta oral durante la infusión isocalórica de glucosa o suero fisiológico. Se compararon las entradas, salidas y balance de nitrógeno, los cambios en el peso corporal y el tamaño del hígado. Los dos glicéridos del 3-hidroxibutirato y la infusión de glucosa, mostraron similar retención de nitrógeno, cambios en el peso corporal y tamaño del hígado. Las ratas que recibieron suero fisiológico retuvieron menos nitrógeno y mostraron menor peso corporal e hígados de menor tamaño. Los datos demostraron que los glicéridos del DL-3-hidroxibutirato no son tóxicos, aportan energía por vía intravenosa y podrían ser utilizados como nutrientes para alimentación parenteral.

L'INSULIN-LIKE GROWTH FACTOR I : MARQUEUR NUTRITIONNEL ET PRONOSTIC AU COURS DE LA CIRRHOSE.

La plupart des paramètres classique de l'évaluation nutritionnelle sont d'un intérêt limité chez le patient cirrhotique. L'IGF-I est régulé par la nutrition et pourrait donc constituer un marqueur de l'état nutritionnel Cependant, son utilisation chez le cirrhotique n'a pas été évaluée.

L'IGF-I sérique et les valeurs de différents paramètres anthropométriques et biologiques ont été déterminés chez 64 patients cirrhotiques hospitalisés avec un suivi clinique de deux ans.

Le Z score de l'IGF-I était de l'ordre de -2,16 ± 1,08 et était corrélé négativement au score de Child Pugh ($p < 0,01$) qui est le plus fiable pour apprécier la sévérité de l'atteinte hépatique. Le Z score de l'IGF-I n'était pas différent entre les patients avec et sans signes de malnutrition énergétique définie par des valeurs de circonférence musculaire brachiale (CMB) et/ou du pli cutané tricipital (PCT) inférieures au 5eme percentile. Il n'y avait pas de corrélation entre le Z score de l'IGF-I et la CMB ou le PCT. Quoique corrélée avec les différentes protéines viscérales, la réduction de l'IGF-I était plus importante et plus fréquente que celle des protéines viscérales. Les patients ayant un Z score de l'IGF-I inférieur à la valeur médiane (-2,5) présentaient un taux de survie à long terme plus faible que ceux avec un Z score supérieur à -2,5 ($p< 0,01$).

Ces résultats indiquent que l'IGF-I sérique n'est pas corrélé avec la malnutrition énergétique chez les patients cirrhotiques, mais qu'il présente un bon caractère prédictif en termes de survie et constitue un marqueur précoce des altérations hépatiques. De nombreux facteurs, pour la plupart reliés à la sévérité de l'atteinte hépatique, pourraient contribuer à la diminution de la concentration de l'IGF-I. Cette pathogénie complexe est sans doute à l'origine du caractère prédictif de ce paramètre.

EVALUATION PÉRIOPÉRATOIRE DU MÉTABOLISME DU GLYCÉROL À L'AIDE D'ISOTOPES STABLES.

Le but de ce travail était d'évaluer les modifications métaboliques pendant et après hystérectomie en s'intéressant plus particulièrement au métabolisme du glycérol. Sept patientes présentant un myome utérin bénin mais sans autres affections ont été inclues dans cette étude. Le renouvellement du glycérol et la production hépatique de glucose ont été mesurés avant et après intervention à l'aide d'isotopes stables ([1,1,2,3,3-^{2}H5]-glycérol, [6,6-^{2}H2]-glucose). Les concentrations de substrats (glycérol, acides gras libres, ß-hydroxybutyrate, glucose, lactate) et d'hormones (insuline, glucagon, cortisol, catécholamines) ont été déterminées avant, pendant et après l'intervention. L'hystérectomie était associée à une augmentation postopératoire du renouvellement du glycérol (6,46 ± 2,44 vs 3,56 ± 1,28 µmol/kg.min; $p < 0,05$). L'amplitude de l'augmentation était corrélée négativement avec l'âge ($r = 0,872$, $p < 0,05$). Les concentrations de glycérol tendaient à augmenter mais de manière non significative en périopératoire. La production hépatique de glucose et la glycémie augmentaient en postopératoire, respectivement de 9,75 ± 1,61 à 12,79 ± 1,15 µmol/kg.min ($p < 0,05$) et de 4,6 ± 0,9 à 6,2 ± 0,9 mmol/l ($p < 0,05$). Les concentrations de cortisol et de catécholamines augmentaient pendant et après l'intervention, tandis que celles d'insuline et de glucagon ne variaient pas. L'accélération de la lipolyse après hystérectomie n'était pas identifiable par la seule mesure des concentrations plasmatiques de glycérol. Ces résultats montrent que l'utilisation des isotopes stables permet une meilleure évaluation des flux métaboliques que des simples dosages statiques de substrats plasmatiques, en particulier en période périopératoire.

UTILISATION D'UN SYSTÈME EXPERT DANS LA PRESCRIPTION DES DIÈTES ENTÉRALES EN CENTRE HOSPITALIER UNIVERSITAIRE.

Un système expert développé sur PC a été mis au point pour la prescription des diètes entérales en fonction des besoins des patients. Deux cent douze patients hospitalisés ont été évalués prospectivement afin de comparer la nature et le coût des diètes définies par le système expert et par l'équipe soignante. Deux cent sept patients ont pu être analysés. L'économie moyenne par patient (± sd) était de 1,18 ± 7,69 $ avec le système expert ($p < 0,023$). Nous évaluons à 27.564 $ par an l'économie que permettrait de réaliser l'utilisation de ce système dans notre hôpital (pour une moyenne de 23.360 jours de nutrition entérale). Nous en concluons que l'utilisation d'un système expert permet une économie réelle pour la prescription des diètes entérales chez les patients hospitalisés.

EICOSANOÏDES ET ÉMULSIONS LIPIDIQUES AU COURS DU SYNDROME DE DÉFAILLANCE RESPIRATOIRE AIGUË (SDRA).

L'administration d'émulsions lipidiques est responsable de modifications de la fonction respiratoire. Bien que ces altérations soient imputables aux effets physiques de l'hyperlipidémie induite par la perfusion sur les échanges gazeux, plusieurs études expérimentales et cliniques suggèrent que les altérations de la fonction respiratoire lors de la perfusion de lipides sont dues aux prostaglandines. Ces dernières sont synthétisées par voie enzymatiques à partir des acides gras essentiels (AGE). Nous avons comparé les effets de deux émulsions lipidiques différant par leur contenu en AGE (20% de triglycérides à chaînes longues contenant 55% d'acide linoléique et 7% d'acide α-linolénique vs un mélange physique à 20% de triglycérides à chaînes moyennes et à chaînes longues contenant 26% d'acide linoléique et 4% d'acide α-linolénique) sur les concentrations plasmatiques d'eicosanoïdes chez des patients présentant un SDRA. Bien que les patients SDRA présentaient des concentrations plasmatiques de prostanoïdes supérieures aux valeurs usuelles, aucune des deux émulsions lipidiques administrées à la vitesse de 2 mg/kg.min n'entraînait de modifications significatives des eicosanoïdes en dehors d'une diminution des la différence artério-veineuse pulmonaire de 6-céto prostaglandine F1α.

ZINC ET CIRRHOSE HÉPATIQUE : ÉVALUATION BIOCHIMIQUE ET HISTOPATHOLOGIQUE.

Une cirrhose hépatique expérimentale a été induite chez l'animal par administration de thioacétamide. Les animaux cirrhotiques ont été répartis en deux groupes, l'un recevant du sulfate de zinc et l'autre servant de contrôle. Les animaux traités ont présenté une restauration des concentrations hépatiques et plasmatiques de zinc et de cuivre. De même, les activités plasmatiques de l'aspartate amino-transférase (ASAT), de l'alanine amino-transférase (ALAT) et de la γ-glutamyl transférase (GGT) et la bilirubinémie diminuaient significativement. L'analyse en microscopie optique montrait une morphologie normale pour la plupart des hépatocytes chez les animaux traités par rapport aux contrôles. Le contenu en fibrine, en réticuline et en collagène, élevé chez les animaux cirrhotiques, diminuait après traitement par le zinc. La coloration à l'acide périodique de Schiff (PAS) montrait la capacité des hépatocytes à stocker du glycogène après traitement par le zinc. Ces résultats indiquent que le zinc pourrait avoir des effets positifs dans le traitement de la cirrhose du foie.

MONO- ET TRIGLYCÉRIDES DE DL-3-HYDROXYBUTYRATE EN NUTRITION PARENTÉRALE : SYNTHÈSE ET ÉVALUATION BIOLOGIQUE PRÉLIMINAIRE CHEZ LE RAT.

Les esters d'acides organiques à chaîne courte semblent potentiellement intéressant en nutrition parentérale (NP). La plupart des glycérides sont insolubles dans l'eau alors que les esters de corps cétoniques présentent une certaine solubilité. Ainsi, les triglycérides de 3-hydroxybutyrate sont hydrosolubles à la différence des triglycérides d'acéto-acétate. Des mono- et triglycérides de 3-hydroxybutyrate ont été synthétisés et nous avons évalué leur toxicité et leur intérêt nutritionnel en NP. Ces deux composés ont une densité énergétique estimée à 19,7 kJ/g (4,7 kcal/g) et sont hydrosolubles. Ils ont été perfusés (113 kJ/j) à des rats pendant 7 jours en même temps qu'une alimentation orale hypocalorique. Des animaux contrôles, pair-fed ont reçu une perfusion isocalorique de glucose ou de sérum physiologique. Les apports les pertes et le bilan d'azote, les variations de poids corporel et la taille du foie ont été comparés entre les groupes. Les deux glycérides de 3-hydroxybutyrate entraînent une rétention d'azote et des variations du poids corporel et de la taille des foies comparables. Les rats recevant du sérum physiologique avaient une rétention d'azote moindre, une perte de poids et une taille des foies diminuée. Ces résultats démontrent que les glycérides de 3-hydroxybutyrate sont dépourvus de toxicité; ils peuvent servir de substrats énergétiques par voie intraveineuse et pourraient être utilisés en nutrition parentérale.

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Zinc and Liver Cirrhosis: Biochemical and Histopathologic Assessment

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ABSTRACT

Experimental liver cirrhosis was produced by administration of thioacetamide. Cirrhotic animals were divided into two groups: one group was given zinc sulphate and the second kept as cirrhotic control. Zinc-treated animals showed a restoration of normal hepatic and plasma zinc and copper levels. Similarly, plasma levels of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl aminotransferase, and total bilirubin decreased significantly. Light microscopic studies showed that most of the hepatocytes appeared normal in zinc-treated as compared with untreated cirrhotic animals. The amount of fibrin, reticulin, and collagen, which was high in the cirrhotic livers, decreased following zinc treatment. Staining with periodic acid Schiff's reagent showed the ability of heptatocytes to store glycogen after zinc treatment. These results revealed that zinc may have some beneficial effect in the treatment of liver cirrhosis. *Nutrition* 1997;13:206–212.

Key words: thioacetamide, liver cirrhosis, liver function test, histopathology, trace elements

INTRODUCTION

Zinc is an essential nutrient with a wide range of biological functions. In experimental and human liver cirrhosis, a decrease in both liver and plasma zinc has been noticed.[1–5] Since zinc plays a major role in the functioning of many enzymes that are concerned with nucleic acid, carbohydrate, and protein metabolism, it is reasonable to believe that changes in zinc metabolism may cause structural and functional abnormalities in the cell.

Zinc is involved in stabilizing the cell membrane and prevents oxidative destruction caused by free radicals.[6–10] Zinc is essential for the proliferation of hepatocytes and plays an important role in glucose metabolism.[11] It was shown that in liver, the activity of ornithine carboxylase, a key enzyme involved in the synthesis of hepatic urea, is decreased in experimental zinc-deficient animals.[12,13] Furthermore, zinc is necessary for the growth and multiplication of cells, and responsible for DNA and RNA synthesis.[14] Zinc is involved in the functioning of more than 300 enzymes.[15,16] It is required for wound healing and skin integrity, as it is involved in the synthesis of proteins such as collagen,[14–16] and important for the functioning of taste, smell, and eyesight[15,17] with a major role in the metabolism of vitamin A.[18] Zinc is required for the activation of macrophages, polynuclear cells, and T lymphocytes, thereby playing a major role in anti-inflammatory actions in maintaining the integrity of the immune system.[19]

Because of the biological significance of zinc and the problems arising from zinc deficiency in liver cirrhosis, studies in our laboratory are mainly focused on the role of zinc in counteracting the cirrhotic changes in humans and animal models. Previously we have shown that the specific activity of superoxide dismutase was significantly decreased in experimental cirrhotic animals and returned to normal after zinc therapy.[20] We recently reported on the normalization of plasma amino acid levels following zinc supplementation in patients with Bilharzial liver fibrosis[21] and in rat models of experimental liver cirrhosis.[22] In another study it was found that zinc administration normalizes the plasma level of zinc

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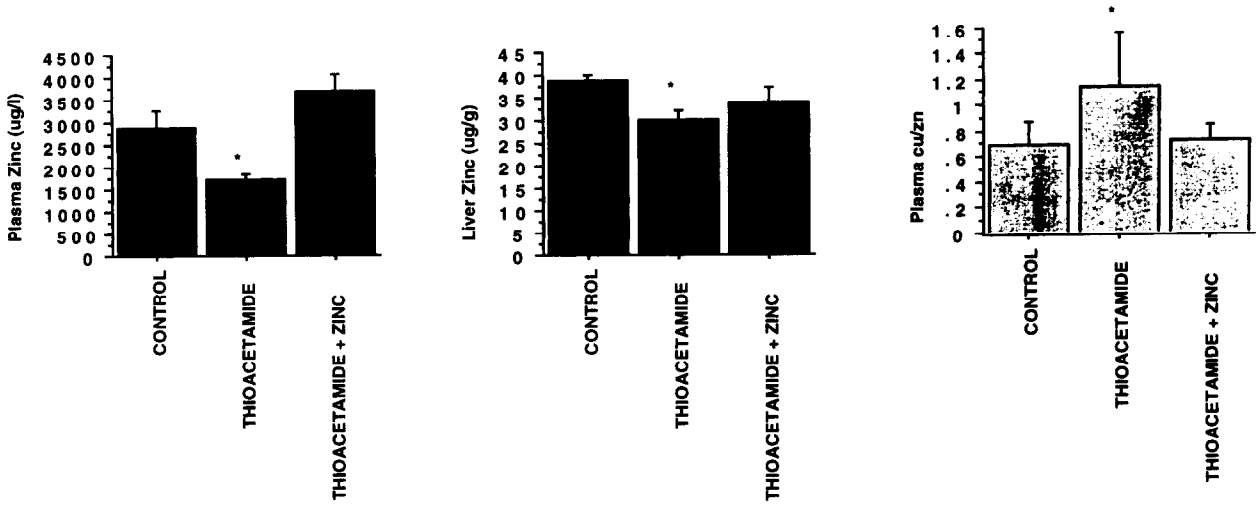

FIG. 1. Levels of plasma zinc, liver zinc, and the plasma Cu/Zn ratio in control, thioacetamide-treated, and thioacetamide/zinc-treated rats (mean ± SE). $*P < 0.01$ compared with control and zinc treated. Cu, copper; Zn, zinc.

and copper, free amino acids, and the ratio of branched-chain amino acids to aromatic amino acids in cirrhotic rats.[23] Moreover, the plasma level of interleukin-8 (IL-8) was significantly reduced following zinc treatment.[24] The present study is focused on the effect of zinc in thioacetamide-induced cirrhotic rats by biochemical and histologic methods.

MATERIALS AND METHODS

Twenty male Wistar rats, locally bred (Bnatin and Kingman Ltd., Grimston Aldboriugh, North Humberside, England), weighing about 200 g were employed in this study. Three animals each were kept in a cage and fed a standard laboratory diet and water ad libitum. The diet consisted of crude oil, crude protein, crude fiber, ash saturated fatty acids, lysine, methionine, cystine, threonine, tryptophan, arginine, calcium, phosphorous, potassium (salts), magnesium, iron (129 mg/kg), copper (12.6 mg/kg), manganese (73.2 g/kg), zinc (35.8 mg/kg), iodine (992.6 g/kg), cobalt (1040.9 g/kg), selenium (the exact amount is not calculated), and vitamins (A, D, E, K, B_1, B_2, B_6, B_{12}).

Experimental liver cirrhosis was produced by administration of thioacetamide (0.3 g/L of tap water) for a period of 28

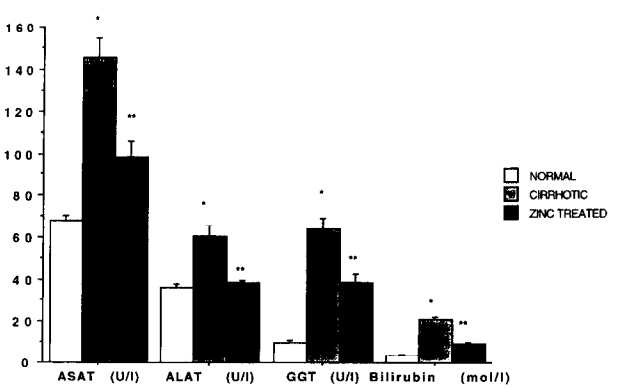

FIG. 2. Effect of zinc treatment on the plasma levels of AST, ALT, GGT, and total bilirubin in thioacetamide-induced cirrhotic rats as compared with normal rats. $*P < 0.001$ compared with control. $**P < 0.001$ compared with cirrhotic. AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase.

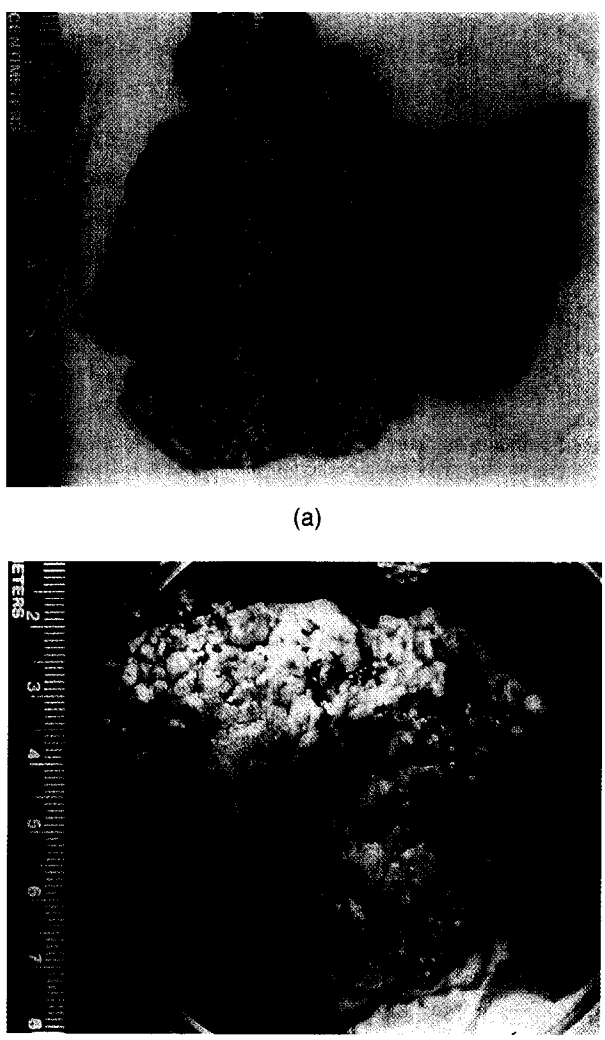

FIG. 3. Macroscopic appearance of cirrhotic (a) and zinc-treated cirrhotic (b) rat liver. The number of micronodules is significantly decreased in liver of zinc-treated animals.

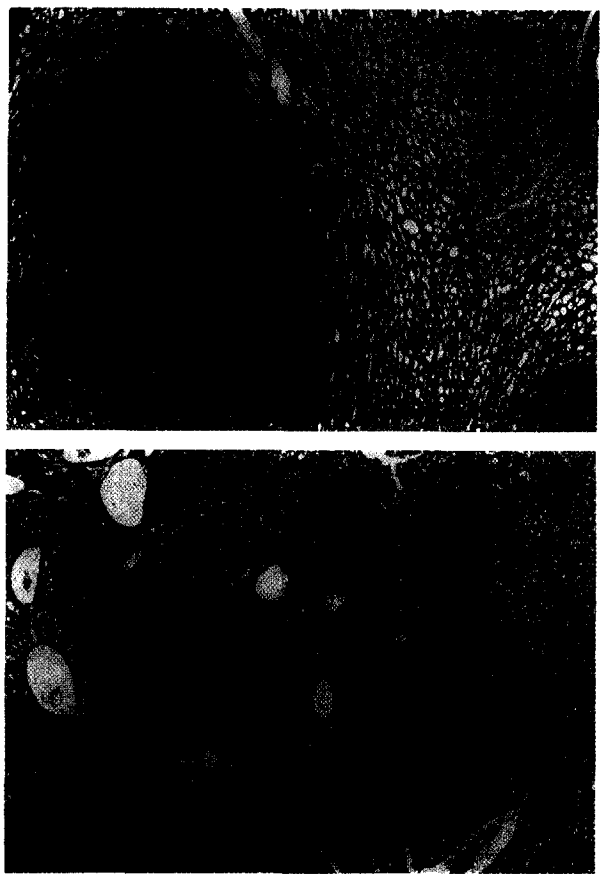

FIG. 4. Histologic characteristics of cirrhotic (a) and zinc-treated cirrhotic (b) rat liver as studied by hematoxylin and eosin staining method. In cirrhotic liver, the normal architecture of the hepatic lobule is completely distorted and the hepatocytes appear abnormal in various respects and many are hypertrophied. Various regions of the cirrhotic liver are filled with many proliferated bile ducts. Following zinc treatment, the number of bile ducts is significantly reduced and many hepatocytes appear to retain their normal characteristics, although some are hypertrophied (a and b × 160).

wk.[25] From the 29th week until the end of the 30th, the animals were fed a standard laboratory diet and water ad libitum. From the 30th week, cirrhotic animals were divided into two groups; one group was subjected to zinc sulphate consisting of 227 mg of zinc[26] dissolved in a liter of tap water. Duration of zinc administration started from 30th week for 14 wk. The second group of rats was given the same diet except that they were not treated with zinc sulphate. In this study a third group of age-matched rats was used as normal controls. All the rats were kept until the end of experimental period (44 wk).

Six animals from each group were used for biochemical and histologic analysis, respectively. Biochemical studies included the determination of the plasma and hepatic level of zinc and copper as well as the liver function tests such as the plasma level of total bilirubin,[27] aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT). For determining the plasma level of total bilirubin, AST, ALT, GGT, zinc and copper, the blood was drawn in metal-free tubes from the abdominal aorta under ether anesthesia. From these animals, approximately 1 g of the liver tissue was kept frozen for determining the hepatic level of copper and zinc. The tissue samples were dissolved in nitric acid and the levels of trace elements were analyzed by atomic absorption spectrophotometry. Levels of AST, ALT, GGT, and total bilirubin were carried out using sera-pak kits (Miles Ltd., Canada) according to manufacturer's instructions.

For histologic studies, the animals were anesthetized with

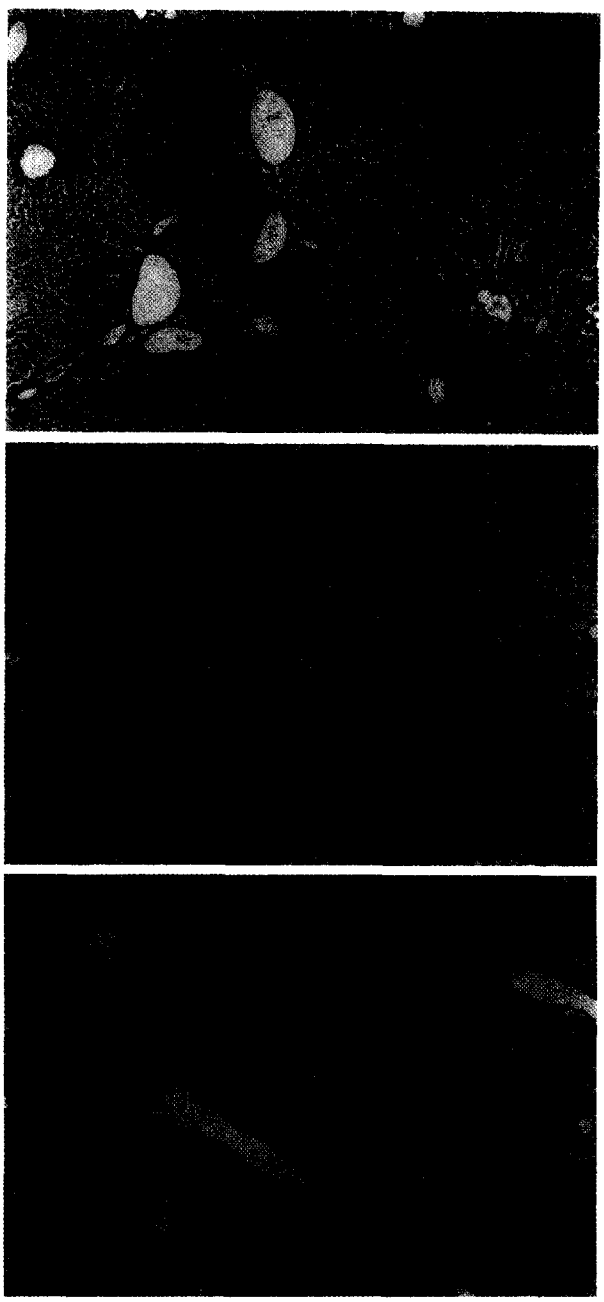

FIG. 5. Histologic localization of collagen by van Gieson's staining method in normal (a), cirrhotic (b), and zinc-treated cirrhotic (c) rats. In normal liver, collagen is mainly located at the periportal regions. The amount of perisinusoidal collagen is very little or almost absent. In cirrhotic liver, collagen is present all over the liver with a heavy deposition around the proliferated bile ducts. Following zinc treatment, the level of collagen in the liver of cirrhotic rats is highly reduced and mainly restricted to the septal regions of the nodules (a, b, and c × 160).

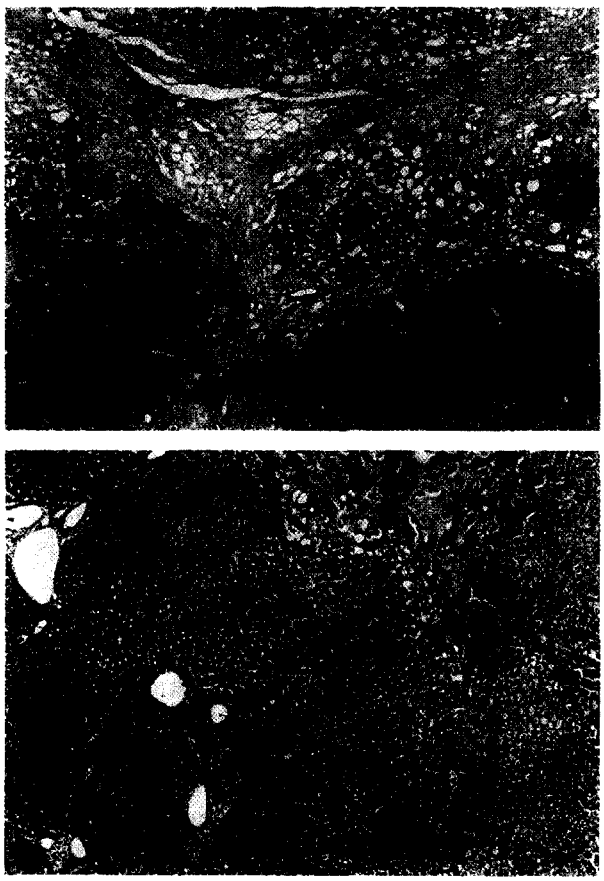

FIG. 6. Histologic localization of the level of collagen by Masson's trichrome method in cirrhotic (a) and zinc-treated (b) rat liver. These results are similar to those demonstrated by van Gieson's staining method (a and b × 160).

sodium pentobarbitol and perfused with cold buffered 4% paraformaldehyde. Following perfusion, pieces of liver tissues were dissected out and kept in the same fixative overnight. The tissues were processed for paraffin sectioning and sections of about 5-μm thickness were taken. In addition to the routine hematoxylin and eosin staining, the sections were stained for collagen (van Gieson's and Masson's trichrome method), reticulin (Gordon and Sweet method), fibrin (MSB trichrome), and glycogen (periodic acid Schiff's [PAS] method).

RESULTS

The concentrations of zinc and copper in the plasma and liver of experimental and control animals are shown in Figure 1. The plasma level of zinc decreased significantly in thioacetamide-treated rats and this level reached above normal levels following zinc treatment. The level of hepatic zinc was also significantly decreased in thioacetamide-induced rats, and increased substantially following the administration of zinc. The ratio between the level of copper and zinc is altered significantly in the plasma and liver of rats after thioacetamide treatment, but returned to normal in both plasma and liver following zinc therapy (Fig. 1). The level of total bilirubin AST, ALT, and GGT (Fig. 2) was elevated in the liver of thioacetamide-treated rats, but decreased significantly following zinc administration. The level of ALT and total bilirubin returned to almost normal after zinc treatment.

Morphologic studies have shown that the cirrhotic liver was enlarged and micro- and macronodules were present on its surface, with a predominance of micronodules (Fig. 3a). However, the liver surface of zinc-treated animals showed a decrease in the number of micronodules as compared with that of the cirrhotic animals (Fig. 3b). Hepatic sections from normal, cirrhotic, and zinc-treated animals were stained with hematoxylin and eosin to study the general histologic architecture. The cirrhotic liver sections showed a massive change in general structure as compared with the normal. In cirrhotic sections the normal lobular architecture was completely distorted and there was an enhanced proliferation of bile ducts (Fig. 4a). Among the proliferated bile ducts, the hepatocytes were organized in patches. Under high magnification, certain hepatocytes undergoing mitosis were also observed. In the sections of the liver from zinc-treated animals (Fig. 4b), there was a significant decrease in the number of bile ducts as compared with the cirrhotic liver (Fig. 4a). In these sections most of the hepatocytes appeared normal, although some were still hypertrophied.

Since the synthesis and accumulation of connective tissue fibers play a major role in the development of cirrhosis, we have compared the level of collagen, reticulin, and fibrin in the liver sections of normal, cirrhotic, and zinc-treated animals. The amount of collagen present in the liver of these three groups of animals was studied by the van Gieson's and the Masson's trichrome methods. The amount of collagen was highly increased in the liver of cirrhotic rats as compared with the normal (Figs. 5a,b, 6a). However, there was a significant decrease in the level of collagen in the liver following zinc treatment (Figs. 5c, 6b). Thus the collagen level in the liver of zinc-treated animals was significantly reduced. Similar changes were observed in the level of fibrin and reticulin as studied by the MSB trichrome method (Fig. 7a,b,c) and the Gordon and Sweet method, respectively (Fig. 8a,b,c). PAS staining method has shown that a significant number of hepatocytes have lost their ability to store glycogen in liver sections of cirrhotic rats (Fig. 9a,b); following zinc treatment, it appeared that most of the hepatocytes have either partially or fully regained their ability to store glycogen (Fig. 9c).

DISCUSSION

The oral administration of thioacetamide produced definite cirrhotic changes in rats. In agreement with the biochemical studies of earlier investigators, we have shown that zinc treatment normalizes the hepatic and plasma level of zinc and copper in cirrhotic rats. In addition, we have shown that following zinc administration, there was a significant decrease in the level of total bilirubin, AST, ALT, and GGT, the indicators of liver function in cirrhotic rats. The results are in agreement with the studies of Vanaja and Gajalakshmi,[27] who showed that zinc normalizes the plasma level of AST, ALT, and alkaline phosphatase in cirrhotic rats in a dose-dependent manner. Therefore, a decrease in the level of these biochemical indicators of liver integrity and function suggests that zinc may be useful in reversing certain experimentally induced changes in cirrhotic rats.

Zinc therapy in experimental liver cirrhosis also normalizes the plasma level of free amino acids and IL-8.[12,13,21,22,24] Administration of zinc inhibits the formation of malondealdehyde, decreases the accumulation of collagen in the liver, and stabilizes the lysosomal membranes in animals exposed to carbon tetrachloride (H. Dashti, unpublished observations).[25] It also inhibits platelet aggregation and is essential for the proliferation of liver cells.[27-29]

In addition to the biochemical changes, we have observed a marked decrease in the level of collagen, reticulin, and fibrin

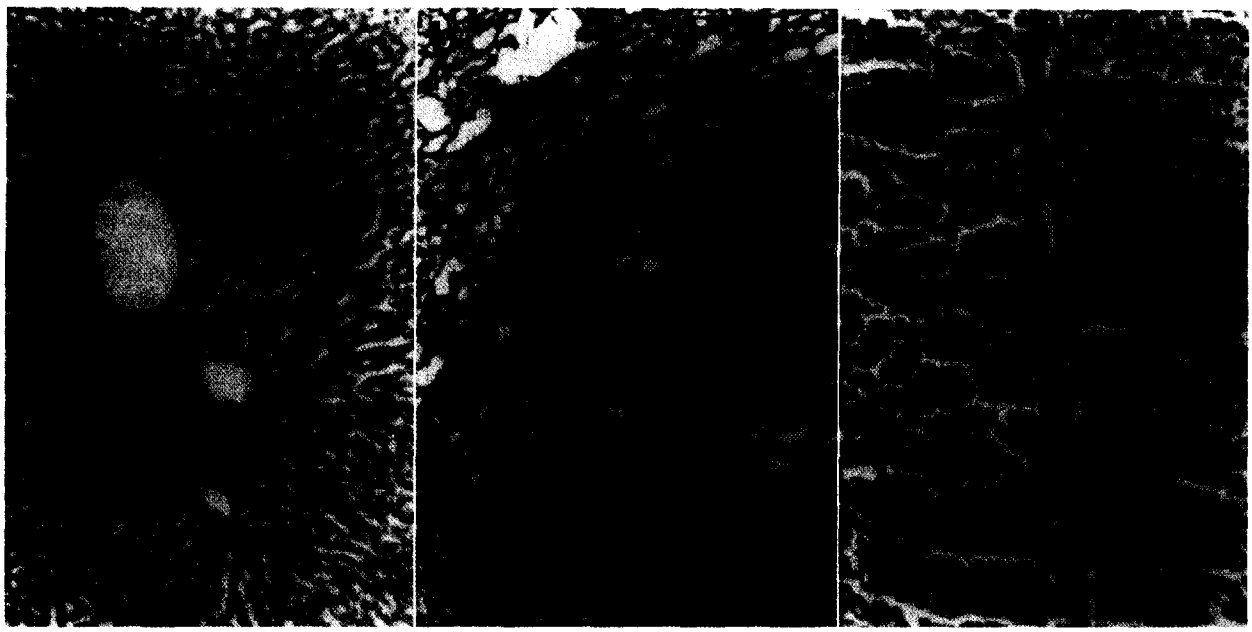

FIG. 7. Demonstration of the amount of fibrin in the liver of normal (a), cirrhotic (b), and zinc-treated cirrhotic (c) rats by MSB trichrome method. The pattern of distribution of fibrin in the liver is similar to that of collagen (see Fig. 3) in all the groups of animal studied, ranging from the presence of a very little amount of perisinusoidal fibrin in the normal rat liver to a heavy deposition of fibrin in the cirrhotic liver, especially around the proliferating bile ducts. The level of fibrin was markedly decreased following zinc treatment and was present mainly at the nodular septa (a, b, and c × 275).

in the liver of zinc-treated cirrhotic rats as compared with that of untreated animals. PAS staining has shown that the hepatocytes in the cirrhotic liver have partially regained the ability to store glycogen.

The present study is in agreement with the earlier works of Saldeen,[30] Ludwig and Chvapil,[6] and Antinen et al.,[25] who also showed changes in certain histologic features of the liver following zinc treatment. Sladeen[30] observed that zinc treatment prevents fatty infiltration and necrosis in liver parenchyma of carbon tetrachloride-treated rats. Ludwig and Chvapil[6] demonstrated that pretreatment of rats with 2 mg of zinc chloride/100 g body weight intraperitoneally for three

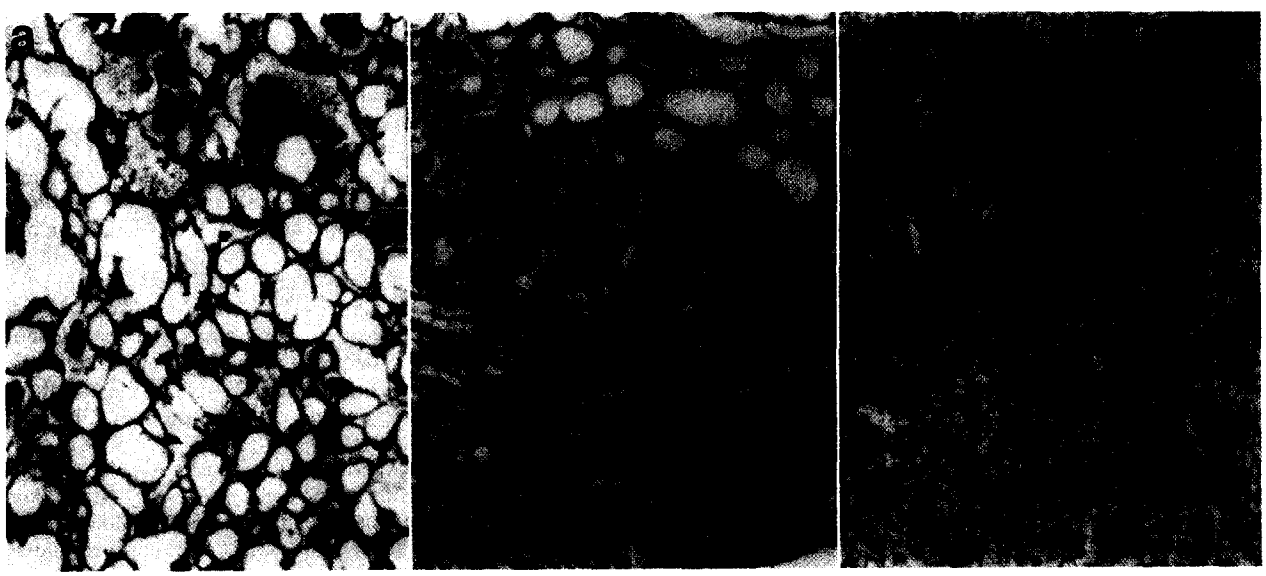

FIG. 8. Histologic analysis of the amount of reticulin in normal (a), cirrhotic (b), and zinc-treated cirrhotic (c) rat liver using the Gordon and Sweet staining method. The amount of reticulin is highly increased in the cirrhotic rat liver as compared with the normal liver. However, in zinc-treated cirrhotic liver there is a marked reduction in the level of reticulin in certain regions as shown in (c), although the localization of reticulin in certain areas of the liver was quite similar to that of the untreated cirrhotic liver (a, b, and c × 275).

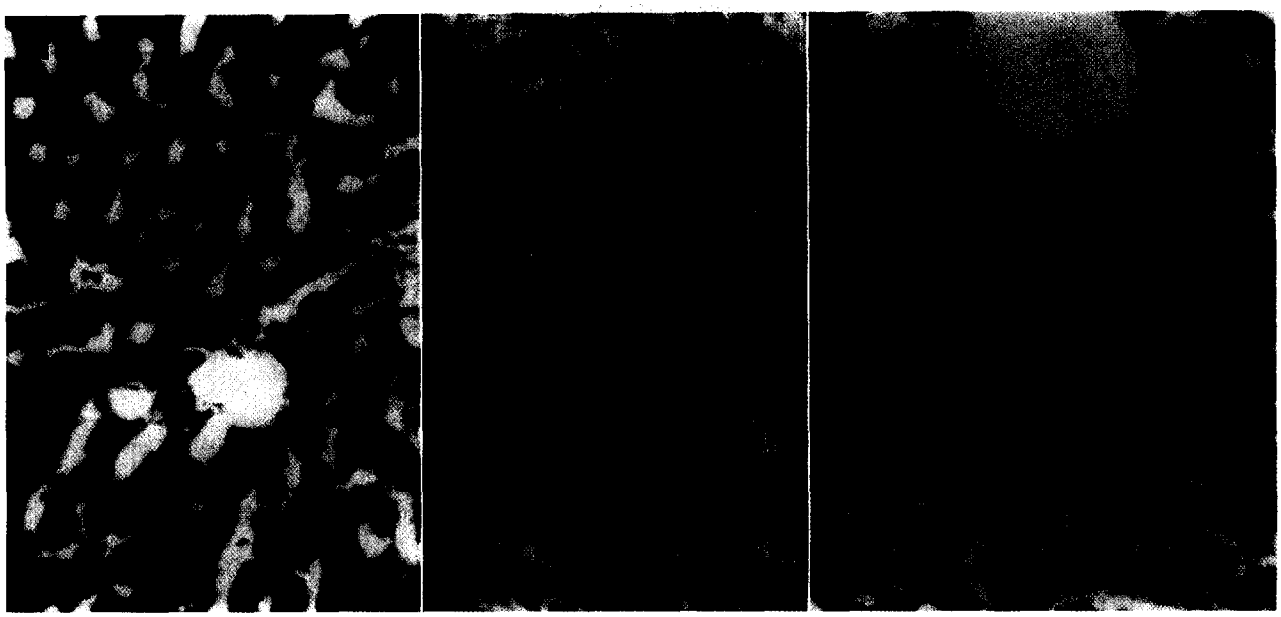

FIG. 9. Histologic analysis on the amount of glycogen present in normal (a), cirrhotic (b), and zinc-treated cirrhotic (c) rat liver using periodic acid Schiff's staining method. In cirrhotic liver, the majority of the hepatocytes do not contain a significant amount of glycogen. Following zinc treatment, the amount of glycogen was retained at its normal level in a number of hepatocytes. (a, b, and c × 275).

consecutive days resulted in a 50% reduction of carbon tetrachloride–induced centrilobular necrosis, along with a reduction in the level of lysosomal enzymes. Antinen and his collaborators[25] found that oral supplementation of zinc in rats has a direct and selective inhibitory effect on carbon tetrachloride-induced collagen accumulation in the liver. Similarly, in agreement with our results, Gimenez et al.[31] found a decrease in the level of collagen in the liver after subjecting the animls to zinc therapy. On the other hand, Gaudio et al.[32] did not observe any significant difference in the collagen content of the liver following zinc supplementation in experimental liver cirrhosis. In their studies they administered zinc together with carbon tetrachloride in contrast to our studies in which thioacetamide was administered for a period of 28 wk followed by a short period when the cirrhotic animals were fed on the same normal diet as the controls. Zinc treatment started at the end of the 30th week for a period of 14 wk. In addition, they used a higher dose of zinc as compared with what we used in our studies. An elevated level of dietary zinc after a threshold value may show some adverse effects.[29] Zinc has the ability to abate the absorption of copper in the intestine; it is also known that excess zinc induces copper deficiency by increasing metallothionin (MT) synthesis in the intestine and MT in turn binds to copper, which is later excreted. Copper deficiency on the other hand leads to a decrease in the activity of copper metalloenzymes such as superoxide dismutase.[30]

The functional role of zinc in ameliorating the symptoms of liver disorders due to cirrhosis has not been understood. However, the wide range of functional roles attributed to zinc support its protective and other beneficial effects in cirrhotic rat models. In addition to the functions mentioned earlier, zinc plays a major role in the synthesis and degradation of collagen, and collagenase was found to be a zinc metalloenzyme.[33,34] Zinc also acts as an effective metal inhibitor for pyrolyl hydroxylase, an enzyme that plays a key role in collagen synthesis.[25] The decrease in the level of zinc observed in experimental cirrhotic rats has a close association with the hepatic pyrolyl hydroxylase activity.[30] This relationship itself may support a rationale for using zinc in the treatment of liver cirrhosis. The mechanism by which zinc inhibits the action of pyrolyl hydroxylase activity is not well understood, but it is believed that zinc may replace iron on the active site of the enzyme, thereby inhibiting its action.[25] It has also been shown that zinc administration may modify collagen accumulation through changes in vitamin A metabolism.[35]

The possible relationship between zinc deficiency and ammonia metabolism also supports a rationale for using zinc in the treatment of cirrhosis. The plasma ammonia level was found to be inversely correlated with liver ornithine transcarbamylase activity, which in turn was correlated with serum and hepatic zinc content.[13] Zinc has a protective effect against membrane peroxidation induced by heavy metals, carbon tetrachloride, and high oxygen tension.[7–10] Lipid peroxidation arising from the reaction of free radicals with lipids is one of the major causes of cellular injury brought about by free-radical attack.[36] Chvapil[7] suggested that zinc interferes with oxidation of nicotinamide adenine dinucleotide phosphate (NADPH), which inhibits the sequence of reactions in lipid peroxidation in the liver and he concluded that dietary zinc controls lipid peroxidation in the liver.

It is not understood whether these beneficial effects of zinc occur through its effect on superoxide dismutase or whether zinc itself has scavenger activity in decreasing lipid peroxidation or it acts in a combination of both. In addition to its role in protecting the cell from free-radical damage, zinc may exert its beneficial effects through various other biological activities as discussed above. In conclusion, zinc appears to reverse certain experimentally–induced cirrhotic changes in the rat. However, more extensive studies are required to understand the exact molecular mechanism of zinc action in the cirrhotic liver.

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