Insulin-Like Growth Factor-I Reverts Testicular Atrophy in Rats With Advanced Cirrhosis

Inma Castilla-Cortazar,[1,5] Maria Garcia,[1,5] Jorge Quiroga,[4] Nieves Diez,[1] Fernando Diez-Caballero,[1,2] Alfonso Calvo,[3] Matias Diaz,[1] and Jesus Prieto[4]

SEE EDITORIAL ON PAGE 783

The pathogenesis of hypogonadism in cirrhosis is not completely understood. The levels of insulin-like growth factor-I (IGF-I), an anabolic factor with trophic actions on testes, are reduced in cirrhosis. This study was undertaken to evaluate whether rats with advanced cirrhosis develop hypogonadism and whether the administration of IGF-I exerts beneficial effects on testicular structure and function. Wistar rats with ascitic cirrhosis induced with CCl_4 were allocated into 2 groups (n = 10, each) to receive recombinant IGF-I (20 $\mu g \cdot kg^{-1} \cdot d^{-1}$, subcutaneously) or vehicle for 3 weeks. Healthy rats receiving vehicle were used as the control group (n = 10). At baseline, both cirrhotic groups showed similar deterioration of liver function tests. Compared with controls, nontreated cirrhotic rats showed decreased serum levels of IGF-I ($P < .05$), reduced testicular size and weight ($P < .001$), and intense histopathological testicular abnormalities, including reduced tubular diameters ($P < .001$), loss of the germinal line ($P < .001$), and diminutions in cellular proliferation, spermatogenesis ($P < .001$), and testicular transferrin expression ($P < .001$). In addition, low serum testosterone ($P < .01$) and high serum LH ($P < .01$) were present in untreated cirrhotic animals. Cirrhotic rats that received IGF-I showed full recovery of testicular size and weight and of all histopathological abnormalities ($P < .001$ to $< .01$ vs. nontreated cirrhotic rats; P = ns vs. controls). Serum levels of sex hormones tended to normalize. In conclusion, IGF-I deficiency may play a pathogenetic role in hypogonadism of cirrhosis. Low doses of IGF-I for a short period of time revert testicular atrophy and appear to improve hypogonadism in advanced experimental cirrhosis. (Hepatology 2000;31:592-600.)

Abbreviations: IGF-I, insulin-like growth factor-I; GH, growth hormone; PCNA, nuclear antigen of cellular proliferation; CO, control healthy group; CI untreated cirrhotic rats; CI + IGF, IGF-treated cirrhotic rats; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

From the Departments of [1]Physiology, [2]Urology, [3]Histology, and [4]Internal Medicine, Liver Unit, University of Navarra, Pamplona, Spain; and [5]Department of Human Physiology, School of Medicine, University of Málaga, Málaga, Spain.

Received September 29, 1999; accepted December 17, 1999.

Supported in part by the program I+D, "Ministerio de Educación y Ciencia" of Government of Spain (SAF 99/0072).

Address reprint requests to: I. Castilla-Cortázar, M.D., Ph.D., Department of Human Physiology, School of Medicine, The University of Málaga, Campus Teatinos, 29080 Málaga, Spain. E-mail: icastill@uma.es; fax: 34-52131650; or J. Quiroga, Department of Internal Medicine, Clínica Universitaria, Avda, Pio XII, 31080 Pamplona, Spain. Fax: 34-48-296785.

0270-9139/00/3103-0008$3.00/0

Hypogonadism is a frequent complication of advanced cirrhosis. This condition is characterized by low testosterone levels and relative hyperestrogenism and is frequently associated with loss of libido, sexual impotence and feminine body habitus in men and amenorrhea in women.[1] It has been attributed to a variety of mechanisms including gonadal toxicity of alcohol, malnutrition, and increased production of estrogens from androgens in peripheral tissues as a result of the existence of portal systemic shunting.[1-8]

On the other hand, insulin-like growth factor–I (IGF-I) is an anabolic hormone produced in different tissues, although the liver accounts for 90% of the circulating hormone that is synthesized in response to growth hormone (GH) stimulation.[9-10] In cirrhosis, the reduction of receptors for GH in hepatocytes and the diminished synthesizing ability of the liver parenchyma cause a progressive fall of serum IGF-I levels. The clinical impact of the reduced production of IGF-I in advanced cirrhosis is largely unknown.[10-14] Recent studies from our laboratory have demonstrated that short courses of treatment with low doses of IGF-I are able to induce marked improvements in nutritional state,[15] intestinal absorption,[16-18] osteopenia,[19] and liver function[20] in rats with experimental cirrhosis. These data suggest that IGF-I deficiency plays a pathogenetic role in several systemic complications occurring in cirrhosis.

It is well known that IGF-I stimulates testosterone synthesis and spermatogenesis.[21-28] Thus, it is possible that its deficiency could contribute to the development of hypogonadism associated with cirrhosis. This study was undertaken to evaluate whether rats with advanced cirrhosis show hypogonadism as assessed by morphological, histopathological, and hormonal parameters, and whether the administration of IGF-I to these rats has beneficial effects on testicular structure and function.

MATERIALS AND METHODS

Induction of Cirrhosis. All experimental procedures were performed in conformity with The Guiding Principles for Research Involving Animals.[29] Cirrhosis was induced as previously described.[15,20] Briefly, male Wistar rats (3 weeks old, 130-150 g) were subjected to CCl_4 inhalation (Merck, Darmstadt, Germany) twice a week for 11 weeks with a progressively increasing exposure time from 1 to 5 minutes. From that time until the 30th week, rats were exposed to CCl_4 once a week for 3 minutes. During the whole period of cirrhosis induction, animals received phenobarbital (Luminal; Bayer Leverkusen, Germany) in the drinking water (400 mg/L). Rats were housed in cages placed in a room with 12-hour light-dark cycle and constant humidity and temperature (20°C). Both food (standard semipurified diet for rodents; B.K. Universal, Sant Vicent del Horts, Spain) and water were given *ad libitum*. Healthy age- and sex-matched control rats were maintained under the same conditions, but receiving neither CCl_4 nor phenobarbital.

Study Design. The treatment was administered the last 3 weeks (27th–30th) of CCl_4 exposure (from day 0 to day 22). In the morning of day 0, animals were weighed and blood samples were drawn from the retro-ocular venous plexus from all rats with capillary tubes (Marienfeld, Mergenheim, Germany) and stored at −20°C until used for analytical purposes. Cirrhotic rats were randomly assigned to receive either vehicle (saline) (Group CI; n = 10) or recombinant human IGF-I (Pharmacia-Upjohn, Stockholm, Sweden) (20 $\mu g \cdot kg^{-1} \cdot d^{-1}$ in 2 divided doses, subcutaneously) (Group CI + IGF; n = 10) for 3 weeks. Control rats (Group CO; n = 10) received saline during the same period. The last dose of IGF-I was administered on day 21 at 6 PM.

In the morning of the 22nd day (at 8 AM), rats were weighed, blood was obtained again and processed as previously indicated, and animals were decapitated. After the abdominal cavity was opened, the liver and the testes were carefully dissected and weighed. A sample from the left major liver lobe and the testes was processed for histological examination (fixed in Bouin's solution). The testicular diameters (AP and LM) were measured in testes previously fixed for histology, using a precision calliper (Mituyoto® [±0.05 mm]).

Liver Histopathology. Bouin-fixed tissues were processed and sections (4-μm) were stained with hemotoxylin-eosin and Masson's trichrome. Livers were scored from 1 to 4, according to histopathological findings: 1) pericentral venous fibrosis; 2), fibrous septa not forming full-shaped regenerative nodules; 3) established cirrhosis with fibrous septa delimiting full-shaped regenerative nodules of variable size (mixed micro-macronodular pattern); and 4) established cirrhosis with fibrous septa delimiting full-shaped regenerative nodules with a difuse micronodular pattern.[30] Pre-established criteria for inclusion of CI animals in the final analysis were the presence of: 1) altered baseline biochemical data of liver function; and 2) in retrospect, histologically proven cirrhosis (score 3 or 4 of the classification) in CCl_4-treated animals.

Testicular Histopathology and Proliferating Cell Nuclear Antigen (PCNA) and Transferrin Immunohistochemistry. For histopathological evaluation of testes, 30 seminiferous tubules from each rat of the 3 groups were blindly evaluated by 2 observers, and the arithmetic mean of the scores was taken as the final result. Transversal sections of seminiferous tubuli were examined and evaluation of histological changes was made using a light-projection microscope (Micro Promar Leitz GMBH, Wetzlar, Germany) at 150× magnification. The following parameters were studied: tubular diameter, quantitation of the presence of the different types of cells in tubuli, presence of peritubular fibrosis, and the number of proliferating cells. For general purposes, hematoxylin-eosin and Masson's trichrome staining were used. Specific techniques for other purposes are specified in the corresponding paragraphs.

Tubular diameters were expressed in micrometers. Changes in tubuli were classified into 5 categories (category I: highest damage to Category V: full normality). Category I: presence of only Sertoli cells; category II: Sertoli cells plus spermatids; category III: Sertoli cells plus spermatids plus spermatocytes; category IV: presence of all kinds of cells, but showing some morphological alterations (*i.e.*, severe vacuolization, aberrant cells); category V: presence of all kinds of cells without morphological alterations. The presence of peritubular fibrosis was evaluated in Masson's trichrome preparations according to the thickness of the staining of collagen deposition surrounding tubuli. Proliferating cells were identified by immunostaining of proliferating cellular nuclear antigen (PCNA) using an avidin-biotin peroxidase method[31] with retrieval of antigen by means of microware irradiation. Specific anti-PCNA antibody (mouse anti-PCNA, clone PC 10; DAKO, Glostrup, Denmark) biotinylated rabbit anti-mouse IgG (DAKO) was used, and the avidin-biotin complex technique (ABC, DAKO kit) was performed. The bound antibodies were visualized by means of 3,3′-diaminobenzidine tetrahydrochloride (SIGMA Chemical Company, St. Louis, MO) with nickel enhancement.[31] Finally, samples were slightly counterstained (10 seconds) in hematoxyilin, dehydrated, and mounted in DPX. Controls were performed by substitution of the primary antibody by Tris-buffered saline (TBS). The number of PCNA-positive cells was recorded. The result was expressed as stained cells per tubuli (arithmetic mean of 30 screened tubuli).

Taking into account all the parameters specified above, an overall score of testicular histopathological damage was adopted according to the following guidelines: Tubular diameter (in μm) scored from 0 to 3 points: >260 = 0 points; from 240 to 259 = 1 point; from 220 to 239 = 2 points; and <219 = 3 points. Cellular counts in tubuli: category I (8 points), category II (6 points), category III (4 points), category IV (2 points), and category V (0 points). The score was obtained by multiplying the number of tubuli in each category by its respective points divided by 30 (the number of tubuli evaluated in each animal). Peritubular fibrosis was scored from 0 (absent or minimal) to 1 (evident). Cellular proliferation (PCNA) was scored from 0 to 3 according to the following criteria: When the number of PCNA-positive cells per tubule was higher than 60, the score was 0. When the number of PCNA-positive cells per tubuli was lower than 60, the score was obtained according to the following formula: (60 − PCNA-positive cells) × 0.05. Therefore, the overall score of histopathological damage ranged from 0 (complete normality) to 15 (full abnormality).

In addition, the expression of transferrin in tubuli was evaluated by immunostaining using similar technique as for PCNA with specific anti-transferrin antibody (obtained from rabbit, RARa/TRf; Nordic Immunological Laboratories, Teknovas, the Netherlands). Transferrin expression was scored from 0 to 4 points. If 30 tubuli expressed transferrin normally all over the germinal epithelium: 0 points. The remaining scores were obtained according to the following formula: (30 − tubuli showing expression of transferrin all over the germinal epithelium) × 0.075.

Analytical Methods. Serum levels of albumin, total proteins, glucose, cholesterol, bilirubin, alkaline phosphatase, transferrin, aspartate transaminase (AST), and alanine transaminase (ALT) were determined by routine laboratory methods using a Hitachi 747 autoanalyzer (Boehringer-Mannheim, Germany). Serum levels of the different hormones were assessed by radioimmunoassay in a GammaChen 9612 Plus (Serono Diagnostics, Rome, Italy) using specific commercial assay systems: total and free testosterone and estradiol-6, Coat-a-Count, DPC (Diagnostic Products Corporation, Los Angeles, CA); rat luteinizing hormone (rLH) and rat follicle-stimulating hormone (rFSH) from Amersham International plc (Little Chalfont, Buckinghamshire, England); IGF-I by extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA).

Statistical Analysis. Data are expressed as mean ± SEM. To assess the homogeneity among the 3 groups of rats, a Kruskal-Wallis test was used, followed by multiple *post-hoc* comparisons using the Mann-Whitney *U* test (2-tailed) with Bonferroni adjustment. A regression model was fitted considering histopathological score, PCNA or transferrin expression scores, and IGF-I plasma concentration as the dependent and independent variables, respectively. Within-groups differences between pre- and posttreatment values were assessed by means of Wilcoxon matched pairs signed rank sum test. $P < .05$ was considered to be statistically significant. Calculations were performed with the SPSSWin v.6.0. program.

RESULTS

At baseline, groups CI and CI + IGF showed similar serum levels of alanine transaminase, aspartate transaminase, glucose, cholesterol, alkaline phosphatase, bilirubin, total proteins, and albumin, all of which were significantly abnormal as compared with those in control rats. No differences were found between cirrhotic groups (Table 1). At the end of the experimental period, all rats from groups CI and CI + IGF showed variable amounts of ascites at the opening of the abdominal cavity and mixed micro-macronodular cirrhosis on liver histopathology.

Testicular Morphology and Morphometry. There was an evident reduction in testicular size and volume in group CI as

TABLE 1. Biochemical Data (day 0) Before Treatment in the Three Experimental Groups

Parameters	Control Rats (CO, n = 10)	Untreated Cirrhotic Rats (CI, n = 10)	Cirrhotic Rats Before IGF-I Treatment (CI + IGF, n = 10)
Aspartate transaminase (IU/L)	35 ± 2	95 ± 18†	101 ± 20†
Alanine transaminase (IU/L)	21 ± 1	51 ± 5*	53 ± 5*
Glycemia (mg/dL)	161 ± 9	133 ± 11*	132 ± 11*
Albumin (g/dL)	3.4 ± 0.1	2.4 ± 0.2†	2.3 ± 0.2†
Total proteins (g/dL)	6.7 ± 0.1	5.7 ± 0.2*	5.7 ± 0.1*
Cholesterol (mg/dL)	97 ± 4	161 ± 26*	164 ± 26*
Alkaline phosphatase (IU/L)	240 ± 17	565 ± 83†	575 ± 96†
Bilirubin (mg/dL)	0.3 ± 0.1	1.1 ± 0.4*	1.1 ± 0.3*

NOTE. Entries are mean ± SEM.
*$P < .05$ and †$P < .01$ vs. CO.

TABLE 2. Body Weight and Testicular Size and Weight in the 3 Experimental Groups (day 22)

	Healthy Control Rats (CO, n = 10)	Untreated Cirrhotic Rats (CI, n = 10)	Cirrhotic Rats Treated With IGF-I (CI + IGF, n = 10)
Body weight (g)	509.9 ± 4.1	416.8 ± 7.3†	445.1 ± 12.6*
Testes (g)	1.69 ± 0.06	0.99 ± 0.16†	1.46 ± 0.07§
(×100 g bw^{-1})	0.33 ± 0.14	0.24 ± 0.35*	0.33 ± 0.013‡
External testicular diameters (mm):			
Longitudinal	19.26 ± 0.28	15.5 ± 0.86†	18.04 ± 0.32‡
Transversal	9.85 ± 0.19	8.28 ± 0.49*	9.43 ± 0.28‡

*$P < .01$; †$P < .001$ vs. CO.
‡$P < .01$; §$P < .001$ CI vs. CI + IGF.

compared with control and CI + IGF groups (Fig. 1). Morphometric study showed a marked and significant reduction in testicular weight (both in absolute values and when it was corrected by body weight) in group CI as compared with controls and the CI + IGF group. Of note, this last group exhibited full recovery of testicular weight, showing no significant differences with respect to controls. Findings regarding longitudinal and transversal testicular diameters were similar to those in testicular weight. Morphometric data in the 3 groups are summarized in Table 2.

Testicular Histopathology. In group CI, histological examination revealed testicular atrophy with marked decrease in tubular diameter (CO: 283 ± 3; CI: 210 ± 17 nm; $P < .001$). Tubular diameter, however, was not significantly different from controls in rats treated with IGF-I (268 ± 4; $P < .005$ vs. group CI and ns vs. controls). Histopathological findings in untreated cirrhotic rats included: vacuolization of Sertoli's cells, loss of germinal line, detached germ cells, reduction of spermatogenesis, presence of abnormal spermatids (multinucleated cells and cells with intense nuclear staining), and peritubular fibrosis. These alterations were much less intense or almost absent in rats receiving IGF-I (see Fig. 2 for morphological comparison among groups). These changes are presented in Table 3 according to a pre-established score (see Materials and Methods).

FIG. 1. Macroscopic appearance of testes from healthy controls (CO), untreated cirrhotic rats (CI), and cirrhotic animals treated with IGF-I (CI + IGF). Reduced volume of testes is appreciated in cirrhotic rats and recovery to normal size in cirrhotic animals treated with IGF-I.

Testicular cellular proliferation, as evaluated by PCNA, was significantly reduced in CI rats, while CI + IGF rats showed values similar to controls (CO: 63 ± 5; CI: 35 ± 27; CI + IGF: 51 ± 5; $P < .01$ CI vs. CO and CI + IGF; P = ns CO vs. CI + IGF groups). Figure 3 shows PCNA immunohistochemistry in the 3 groups. Taking together all the morphological data, a score of histopathological testicular damage was established (see Materials and Methods). Score values were: CO: 1.18 ± 0.07; CI: 7.41 ± 1.61 ($P < .001$ vs. CO); and CI + IGF: 2.09 ± 0.12 ($P < .005$ vs. CI and ns vs. CO), demonstrating severe testicular damage in CI rats and reversion to normal histology in CI + IGF rats.

Testicular transferrin, a marker of the integrity of the hemato-testicular barrier,[32] was evaluated from 0 to 4 points by immunohistochemistry in testicular slices (see Materials and Methods). Transferrin expression was decreased in CI rats (1.83 ± 0.43 points; $P < .001$ vs. groups CO and CI + IGF) as compared with controls (3.79 ± 0.05 points) and with cirrhotic rats receiving IGF-I (3.60 ± 0.05 points). The last 2 groups showed similar values (Fig. 4). A close inverse correlation was found between testicular transferrin expression and histopathological testicular score ($r = -.95$; $P < .001$).

Pituitary-Gonadal Axis. Nontreated cirrhotic rats showed hypogonadism as manifested by a significant reduction of serum levels of both total and free testosterone serum levels in association with significant increases of LH and estradiol/testosterone ratio and decrease of FSH, as compared with control rats (Table 4). However, as shown in Table 4, in the group of cirrhotic rats treated for 3 weeks with low doses of IGF-I, there was a tendency for the levels of free and total testosterone, estradiol/total testosterone ratio, and LH concentration to move toward normal values, but without reaching significant differences as compared with controls and untreated cirrhotic rats.

Serum Levels of IGF-I. At the time of killing (day 22), cirrhotic rats showed a significant decrease of serum IGF-I levels as compared with controls (CO: 676 ± 46; CI: 449 ± 111 ng/mL; $P < .05$), while CI + IGF rats showed values significantly higher than those in the other 2 groups (966 ± 70 ng/mL; $P < .01$ vs. group CO and $P < .001$ vs. group CI).

Correlation Between Serum IGF-I, Liver Function, and Testicular Histopathology. An inverse correlation was found between serum levels of albumin at the end of the treatment and the

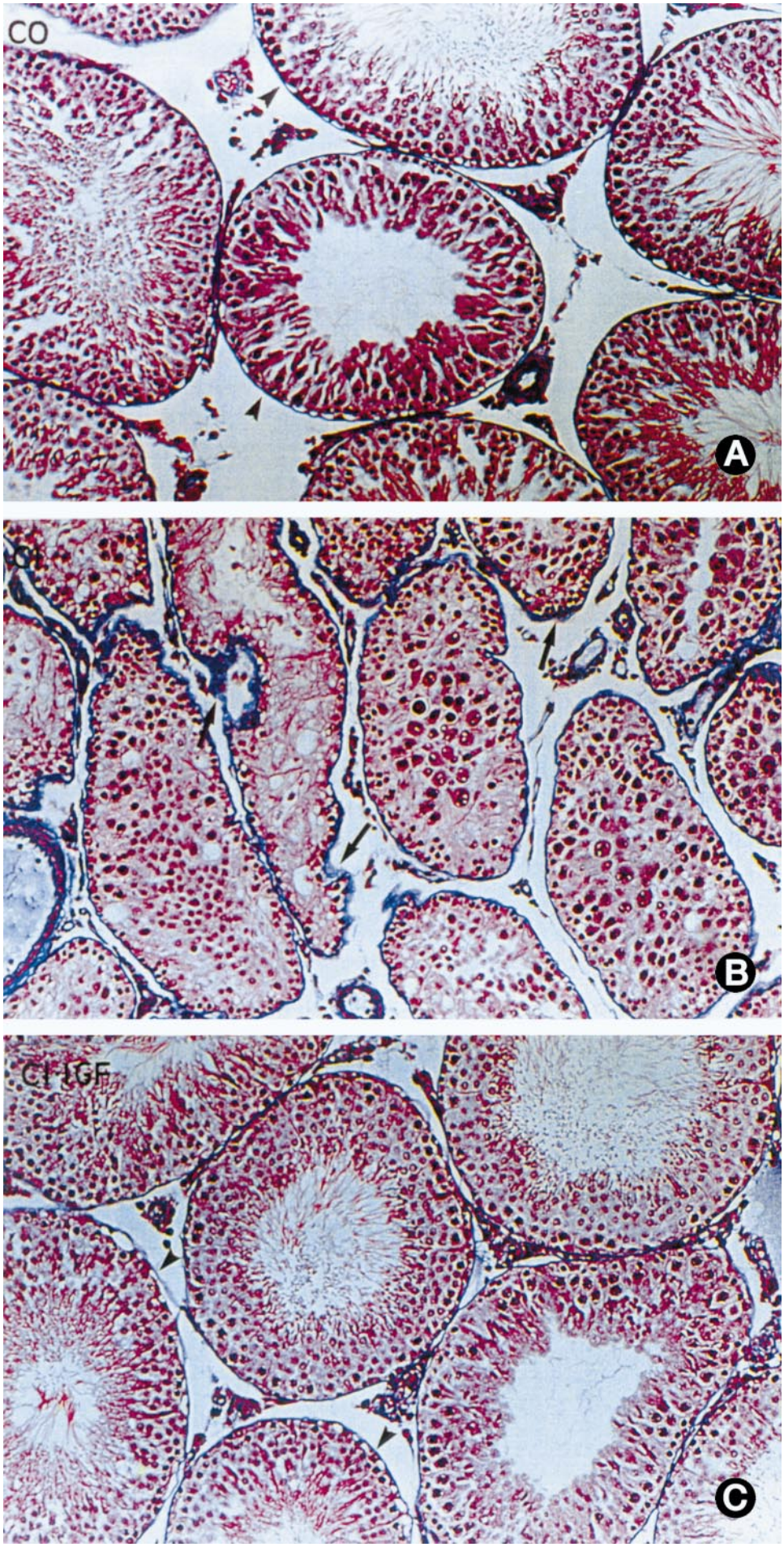

FIG. 2. Microscopy of testes. Testicular histological section of normal rat (CO) demonstrating active spermatogenesis in normal-size seminiferous tubuli with thin basement membranes and minimal peritubular fibrosis (*arrowheads*). Leydig cells are scarce, being widely separated by seminiferous tubuli. Seminiferous tubuli in testes from untreated cirrhotic animals (CI) appear seriously damaged. These animals show a decrease of tubular diameter, vacuolization on germinal epithelium, loss of germinal line, total or partial reduction of spermatogenesis, presence of abnormal spermatids (multinucleated cells, and cells with an intense stained nuclei), and increased collagen deposition (*arrow*). Cirrhotic rats treated with IGF-I (CI + IGF) show reversal of all these alterations. (Masson's stain; original magnification ×150.)

TABLE 3. Cellular Analysis

Category	I (number of tubules)	II (number of tubules)	III (number of tubules)	IV (number of tubules)	V (number of tubules)
Controls (CO) (n = 10) × 30 tubuli	0	0	0	5	295
Untreated cirrhotic rats (CI) (n = 10) × 30 tubuli	26	14	63	57	140
IGF-treated cirrhotic rats (CI + IGF) (n = 10) × 30 tubuli	0	0	0	20	280
Statistical analysis	$P < .001$ CI vs. other groups	$P < .001$ CI vs. other groups	$P < .001$ CI vs. other groups	$P < .001$ CI vs. CO $P < .01$ CI vs. CI + IGF	$P < .001$ CI vs. CO $P < .01$ CI vs. CI + IGF P = ns CO vs. CI + IGF

NOTE. Thirty seminiferous tubuli were examined in each preparation. The table summarizes the number of tubuli in each category: category I = only Sertoli's cells; category II = I + spermatids; category III = II + spermatocytes; category IV = all types of cell but with some alterations; category V = all types of cells with normal features.

histopathological score of testicular damage ($r = -.718$; $P < .001$), suggesting a relationship between liver function and gonadal dysfunction in cirrhotic animals. Besides, a significant direct correlation ($r = .735$; $P < .001$) was present between IGF-I plasma concentrations and serum levels of albumin at the end of the treatment (day 22) in both untreated and IGF-treated cirrhotic animals.

Of interest, a close inverse correlation was observed between IGF-I serum levels and histopathological score of testicular damage in both cirrhotic groups ($r = -.824$; $P < .001$). Also, a significant direct correlation was found between IGF-I levels and both testicular PCNA ($r = .89$; $P < .001$) and testicular transferrin expression ($r = .97$; $P < .001$), suggesting that IGF-I deficiency may play a role in testicular atrophy associated with advanced cirrhosis.

DISCUSSION

This study demonstrates that rats with advanced CCl_4-induced cirrhosis show a severe testicular atrophy and gonadal insufficiency. In addition, the treatment of these animals during 21 days with low doses of IGF-I restores structural testicular integrity and tends to normalize testicular hormonal function.

The occurrence of testicular atrophy and gonadal dysfunction in advanced cirrhosis is a well-known clinical event.[1-8] Both testicular histopathological abnormalities and low levels of serum testosterone have been described in previous years in patients with alcoholic and nonalcoholic cirrhosis.[1-8] Data regarding experimentally induced gonadal dysfunction associated with cirrhosis are, however, scarce. Our data show a severe testicular damage as manifested by macroscopic testicular atrophy and a variety of histopathological abnormalities that include alterations in tubular diameters, presence of aberrant cells in tubular lumen, peritubular fibrosis, loss of the germinal line, and a marked reduction in cellular proliferation. These alterations resemble those found in necropsic studies in alcoholic cirrhotics[2] and those reported in experimental models of testicular damage, such as chronic testicular ischemia.[33]

The mechanisms reponsible for the alterations described in this article are not fully understood. The increased levels of serum LH associated to a significant reduction of total and free serum testosterone defines a picture of primary hypogonadism, thus ruling out hypothalamic-pituitary dysfunction as the responsible mechanism. In cirrhosis caused by either hemochromatosis or alcohol intake, testicular damage is secondary, at least in part, to testicular toxicity caused by iron deposition and metabolic effects of alcohol, respectively.[3,5,6] However, in cirrhosis secondary to other etiologies, hypogonadism also occurs.[1,2,7] Cirrhosis produces two major pathophysiological events: hepatocellular insufficiency as a result of a progressive reduction of liver mass, and portal-systemic shunting owing to the development of portal hypertension. Van Thiel et al. proposed that portal-systemic shunting may contribute to hypogonadism present in cirrhotic patients as a result of the conversion of weak adrenal androgens and estrogens bypassing the liver to estrogens by aromatization in peripheral tissues.[1] In this way, a recent study published by Zaitoun et al.[34] shows that portacaval shunt in rats induces testicular atrophy by reduction in mitosis, maturation arrest, and increased apoptosis of the germinal epithelium. Because these animals were not cirrhotic and thus had normal hepatic function, these results support a major role for portal-systemic shunting in the induction of testicular damage. Although not evaluated in our study, portal-systemic shunting has been demonstrated in the CCl_4 model of cirrhosis,[34] and therefore could have contributed to testicular findings in this article.

On the other hand, no factor has been described directly linking hepatocellular insufficiency and gonadal dysfunction in cirrhosis. Patients with advanced cirrhosis are usually malnourished,[1,7,35] a condition associated with hypogonadism.[7] This factor may contribute to hypogonadism in this study, because cirrhotic rats showed reduced body weight. Indeed, we have previously demonstrated the occurrence of malnutrition in cirrhotic rats and its improvement after IGF-I treatment.[15-18] However, there is no reported association between malnutrition and testicular atrophy, with the only exception being patients with acquired immune deficiency syndrome with chronic infections, orchites, sexually transmitted diseases, and chemotherapy.[40]

In addition, the inverse significant correlation observed in cirrhotic rats between serum albumin and the histopathological score of testicular damage suggests a relationship between testicular state and the hepatic biosynthetic capability. It is possible, therefore, that some substance or substances produced by the liver might be important for testicular integrity. A good candidate for this function would be IGF-I. However, a parallel amelioration in albumin levels and testicular damage related to an improvement in malnutrition by the effect of IGF-I cannot be excluded as an alternative explanation for this correlation.

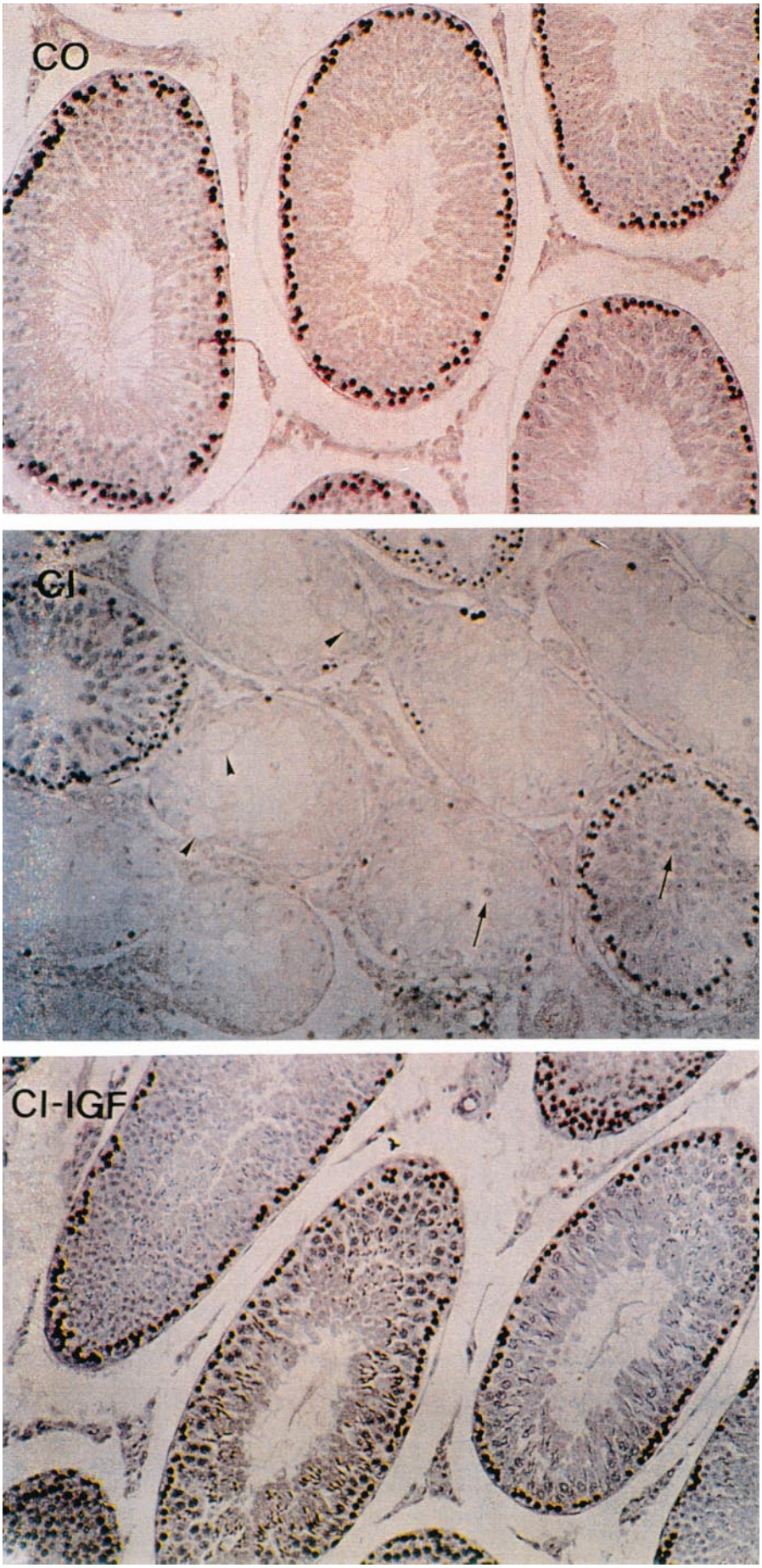

FIG. 3. Study of proliferative activity, assessed by PCNA immunostaining. Reduced or absent (*arrowheads*) cellular proliferation in untreated cirrhotic group (CI) and a normal germinal line in IGF-I–treated cirrhotic rats (CI + IGF) can be observed.

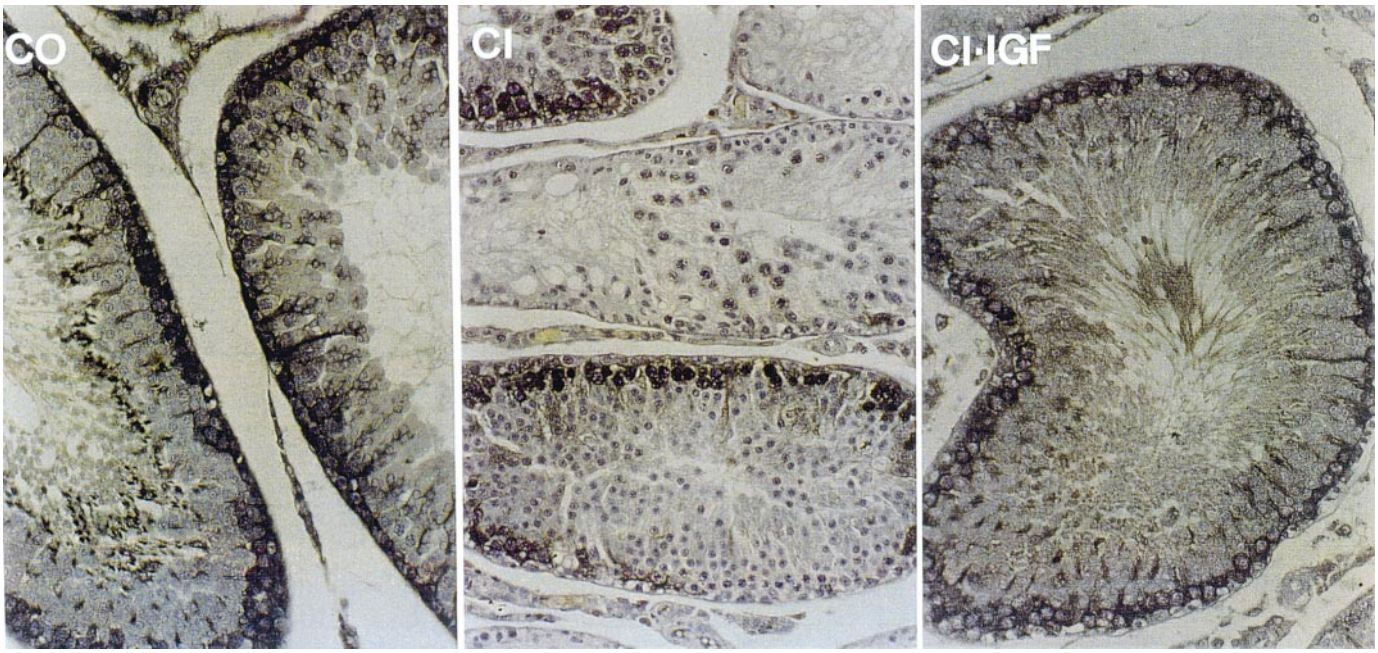

FIG. 4. Immunohistochemistry for testicular transferrin in seminiferous tubuli. Transferrin immunostaining can be seen at the level of Sertoli cells and in germ cells in normal rats (CO) and in cirrhotic rats treated with IGF-I (CI + IGF), but absent transferrin immunostaining is observed in untreated cirrhotic rats (CI).

Until now, no relationship has been demonstrated between IGF-I deficiency and testicular damage in cirrhosis. Advanced cirrhosis leads to a progressive decrease of serum IGF-I levels because of the combination of a reduction of hepatic receptors for GH in the cirrhotic liver and the declining liver function.[10-17] In fact, our cirrhotic rats showed significantly reduced levels of IGF-I. Moreover, we have previously demonstrated that cirrhosis induces changes on the serum profile of IGF-I binding proteins, and specifically, it increases IGF-I binding protein 3 levels.[15] This binding protein reduces the release of IGF-I to tissues. Thus, the composite effect of the reduction of total IGF-I and its increased sequestration by this binding protein could lead to a marked reduction of IGF-I bioavailability in cirrhosis. The administration of IGF-I was followed by a marked increase in IGF-I levels in the treated group 16 hours after the injection on the last day of the study. Although IGF-I half-life in rats is reported to be 3 to 6 hours,[43-45] it has not been measured in cirrhotic animals, and therefore, an increased IGF-I half-life cannot be excluded in these rats.

TABLE 4. The Pituitary-Gonadal Axis (on day 22) in the Three Experimental Groups

	Healthy Control Rats (CO, n = 10)	Untreated Cirrhotic Rats (CI, n = 10)	IGF-I– Treated Cirrhotic Rats (CI + IGF, n = 10)
Total testosterone (ng/dL)	61 ± 13	10 ± 5†	33 ± 15
Free testosterone (pg/mL)	2.67 ± 0.79	0.36 ± 0.17*	0.81 ± 0.42
Estradiol (pg/mL)	9.4 ± 1.6	11.7 ± 3.9	9.5 ± 1.9
Estradiol/total testosterone	0.3 ± 0.1	36.2 ± 13.5*	18.8 ± 10.5
LH (ng/mL)	3.3 ± 0.3	4.4 ± 0.3†	4.0 ± 0.2
FSH (ng/mL)	27.7 ± 1.8	22.2 ± 1.5*	23.6 ± 1.1

*$P < .05$, †$P < .001$ CO vs. CI group.

Because IGF-I is a well-recognized trophic factor for testis, its deficiency could be a contributing factor to testicular damage in cirrhosis. The close inverse and significant correlation between serum IGF-I and parameters of testicular damage observed in this study supports this contention. A major finding in this study was the full recovery of testicular structure after a 21-day course of low doses of IGF-I. No significant differences were observed between control rats and cirrhotic rats treated with IGF-I regarding data of testicular morphometry and histopathological findings. All these parameters showed significant improvement when compared with those of untreated cirrhotic rats. The elucidation of the mechanisms by which IGF-I exerts these beneficial effects requires further investigation. The preservation of the liver function by IGF-I is a possible factor involved.

In a previous study, we showed that parameters of liver function (*i.e.*, serum albumin, clotting factors, clotting tests, and bilirubin) were significantly improved in cirrhotic rats after a treatment such as that used in this study.[20] However, the recovery of the testes seems to be disproportionate to the amelioration of liver function, because in the model of advanced cirrhosis used in the present study, the animals remained cirrhotic and ascitic after completion of the treatment. Similarly, a reduction of porto-systemic shunting capable of restoring testicular structure to normal also seems unlikely, although it might play a contributory role. A direct effect of IGF-I on testes seems to be the most important factor to explain our findings. This idea is supported by the existence of receptors for IGF-I in Sertoli cells, germ cells, and Leydig cells,[22,23,27,28] and by findings demonstrating a direct effect of IGF-I on testes.[21,24,26] Patients with Laron dwarfism, a condition of IGF-I deficiency caused by the absence of receptors for GH, show an increase in testicular

size and serum testosterone levels following treatment with exogenous IGF-I.[38] Stopping IGF-I administration led to a return of both parameters to the pretreatment situation, indicating a specific effect of IGF-I. In mouse, IGF-I gene null homozygous mutation led to a reduced testicular size and infertility.[39] These mice show developmental delay of Leydig cells, and reduced *in vitro* production of testosterone by testicular parenchyma under basal and LH-stimulated conditions.[39] It has also been reported that FSH-induced aromatization of androgens to estrogens in rat Sertoli cells in culture is markedly inhibited by IGF-I treatment through the reduction of aromatase mRNA.[41] Whether this effect of IGF-I might also take place in peripheral tissues remains to be investigated.

In addition, other metabolic functions of Sertoli cells are also influenced by IGF-I.[42] Of interest, the recovery of transferrin expression in Sertoli cells observed in our study suggests a role of IGF-I in maintaining the integrity of the hematotesticular barrier.[32,37] Although all these data indicate that IGF-I exerts potent trophic and hormonogenetic effects on testis, the mechanisms by which IGF-I causes such dramatic changes in testicular structure and function in cirrhotic rats as those demonstrated in this work require further investigation. In our study, however, the recovery of the pituitary-gonadal axis in cirrhotic rats receiving IGF-I was partial. It seems reasonable to think that full recovery could possibly be achieved with a more prolonged treatment, because a normal hormonal production should follow the structural recovery.

In summary, our data suggest that IGF-I deficiency may play a critical role in testicular atrophy and hypogonadism of cirrhosis, and that exogenous administration of this anabolic factor may be useful for the treatment of these alterations in cirrhotic patients.

Acknowledgment: The authors express their gratitude to Dr. Dan Edwall, Pharmacia & Upjohn, Stockholm, Sweden, for generously granting the rhIGF-I used in this study. They are also deeply indebted to the "Real Academia de Medicina de Cataluña" (Barcelona) and the grants from Mrs. C. Alonso-Borrás, Mr. J. Celaya, "Fundación Echébano," and "Fundación C. Regojo."

REFERENCES

1. Van Thiel DH. Endocrine function. In: The Liver: Biology and Pathobiology. Arias, Boyer, Fausto, et al, eds. New York: Raven Press, 1982:717-912.
2. Van Thiel DH, Gavaler JS, Slone FL, Cobb F, Smith WI, Bron KM, Lester R. Is feminization in alcoholic men due in part to portal hypertension? a rat model. Gastroenterology 1980;78:81-91.
3. Bennett HS, Baggenstoss AH, Butt H. The testis, breast and prostate of men who die of cirrhosis of the liver. Am J Clin Pathol 1950;20:814-828.
4. Van Steenbergen W. Alcohol, liver cirrhosis and disorders in sex hormone metabolism. Acta Clin Belg 1993;48:269-283.
5. Galvao-Teles A, Monteiro E, Gavaler JS. Gonadal consequences of alcohol abuse: lessons from the liver. HEPATOLOGY 1986;6:123-128.
6. Guechot J, Vaubourdolle M, Ballet F. Hepatic uptake of sex steroids in men with alcoholic cirrhosis. Gastroenterology 1987;92:203-207.
7. Bannister P, Handley T, Chapman C, Losowsky MS. Hypogonadism in chronic liver disease: impaired release of luteinising hormone. Br J Med 1986;293:1191-1193.
8. Pajarinen JT, Karhunden PJ. Spermatogenic arrest and Sertoli cell-only syndrome-common alcohol-induced disorders of the human testis. Int J Androl 1994;17:292-299.
9. Sara VR, Hall K. Insulin-like growth factor-I and their binding proteins. Physiol Rev 1990;70:591-613.
10. Schimpf RM, Lebrec D, Donadieu M. Somatomedin production in normal adults and cirrhotic patients. Acta Endocrinol 1977;86:355-362.
11. Hattori N, Kurahachi H, Ikekubo K, Ishihara T, Moridera K, Hino M, Saiki Y, et al. Serum growth hormone-binding protein, insulin-like growth factor-I, and growth hormone in patients with liver cirrhosis. Metab Clin Exp 1992;41:377-381.
12. Møller S, Becker U, Juul A, Skakkebæk NE, Christensen E, EMALD Group. Prognostic value of insulin-like growth factor-I and its binding proteins in patients with alcohol-induced liver disease. HEPATOLOGY 1996;23:1073-1078.
13. Møller S, Gronbaek M, Main K, Becker U, Skakkebaek NE. Urinary growth hormone (U-GH) excretion and serum insulin-like growth factor 1 (IGF-1) in patients with alcoholic cirrhosis. J Hepatol 1993;17:315-320.
14. Caufriez A, Reding P, Urbain D, Goldstein J, Copinschi G. Insulin-like growth factor-I: a good indicator of functional hepatocellular capacity in alcoholic liver cirrhosis. J Endocrinol Invest 1991;14:317-321.
15. Picardi A, Costa de Oliveira A, Muguerza B, Tosar A, Quiroga J, Castilla-Cortázar I, Santidrián S, et al. Low doses of insulin-like growth factor-I improve nitrogen retention and food efficiency in rats with early cirrhosis. J Hepatol 1997;24:267-279.
16. Castilla-Cortázar I, Prieto J, Urdaneta E, Pascual M, Nuñez M, Zudaire E, García M, Quiroga J, Santidrián S. Impaired intestinal sugar transport in cirrhotic rats: correction by low doses of insulin-like growth factor I. Gastroenterology 1997;113:1180-1187.
17. Castilla-Cortázar I, Picardi A, Ainzua J, Urdaneta E, Tosar A, García M, Quiroga J, et al. Effect of insulin-like growth factor I on in vivo intestinal absorption in cirrhotic rats. Am J Physiol 1999;276:G637-G642.
18. Pascual M, Castilla-Cortázar I, Urdaneta E, Quiroga J, Prieto J, Santidrián S. Effect of insulin-like growth factor-I (IGF-I) on the jejunal transport of L-leucine and L-glutamic acid in liver cirrhotic rats. FASEB J 1998;12:89A.
19. Cemborain A, Castilla-Cortázar I, García M, Quiroga J, Muguerza B, Picardi A, Santidrián S, et al. Osteopenia in rats with liver cirrhosis: beneficial effects of IGF-I treatment. J Hepatol 1998;28:122-131.
20. Castilla-Cortázar I, García M, Muguerza B, Pérez R, Quiroga J, Santidrián S, Prieto J. Hepatoprotective effects of insulin-like growth factor-I in rats with carbon-tetrachloride-induced cirrhosis. Gastroenterology 1997;113:1682-1691.
21. Tajima Y, Watanabe D, Koshimizu U, Matsuzawa T, Nishimune Y. Insulin-like growth factor-I and transforming growth factor-alpha stimulate differentiation of type A spermatogonia in organ culture of adult mouse cryptorchid testes. Int J Androl 1995;18:8-12.
22. Spiteri-Grech J, Weinbauer GF, Bolze P, Chandolia RK, Bartlett JM, Nieschlag E. Effects of FSH and testosterone on intratesticular insulin-like growth factor-I and specific germ cell populations in rats treated with gonadotrophin-releasing hormone antagonist. J Endocrinol 1993;137:81-89.
23. Zhou J, Bondy C. Anatomy of the insulin-like growth factor system in the human testis. Fertil Steril 1993;60:897-904.
24. Dubois W, Gallard GV. Culture of intact Sertoli/germ cell units and isolated Sertoli cells from squalus testis. II. Stimulatory effects of insulin and IGF-I on DNA synthesis in premeiotic stages. J Exp Zool 1993;267:233-244.
25. Moore A, Morris ID. The involvement of insulin-like growth factor-I in local control of steroidogenesis and DNA synthesis of Leydig cells in the rat testicular interstitium. J Endocrinol 1993;138:107-114.
26. Grizard G. IGF(s) and testicular function. Secretion and action of IGF-I on Leydig cells. Contracept Fertil Sex 1994;22:551-555.
27. Lin T, Wang D, Nagpal ML, Shimasaki S, Ling N. Expression and regulation of insulin-like growth factor-binding protein 1,2,3 and 4 messenger ribonucleic acids in purified rat Leydig cells and their biological effects. Endocrinology 1993;132:1898-1904.
28. Rappaport MS, Smith EP. Insulin-like growth factor (IGF) binding protein 3 in the rat testis: follicle-stimulating hormone dependence of mRNA expression and inhibition of IGF-I action on cultured Sertoli cells. Biol Reprod 1995;52:419-425.
29. The Guiding Principles for Research Involving Animals. National Academy of Sciences and published by the National Institute of Health (NIH), revised in 1991.
30. Fujiwara K, Ogata I, Otha Y, Hayashi S, Mishiro S, Takatsuki K, Sato Y, et al. Decreased collagen accumulation by a prolyl hydroxylase inhibitor in pig serum-induced fibrotic rat liver. HEPATOLOGY 1988;8:804-807.
31. Shu SY, Ju G, Fan LZ. The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. Neurosci Lett 1988;85:169-171.
32. Skinner MK, Cosand WL, Griswold MD. Purification and characterization of testicular transferrin secreted by rat Sertoli cells. Biochem J 1984;218:313-320.

33. Santamaria L, Martin R, Codesal J, Paniagua R. Myoid cell proliferation in rat seminiferous tubules after ischaemic testicular atrophy induced by epinephrine. Morphometric and immunohistochemical (bromo-deoxyuridine and PCNA) studies. Int J Androl 1995;18:13-22.
34. Zaitoun AM, Apelqvist G, Wikell C, AL-Mardini H, Bengtsson F, Record CO. Quantitative studies of testicular atrophy following portacaval shunt in rats. HEPATOLOGY 1998; 28:1461-1466.
35. Smanik EJ, Mullen KD, Giroski WG, McCullough AJ. The influence of portacaval anastomosis on gonadal and anterior pituitary hormones in a rat model standardized for gender, food intake, and time after surgery. Steroids 1991;56:237-241.
36. Mezey E. Liver disease and nutrition. Gastroenterology 1978;74:770-783.
37. Itoh N, Nanbu A, Tachiki H, Akagashi K, Nitta T, Mikuma N, Tsukamoto T, et al. Restoration of testicular transferrin, insulin-like growth factor I (IGF-I), and spermatogenesis by exogenously administered purified FSH and testosterone in medically hypo-physectomized rats. Arch Androl 1994;33:169-177.
38. Laron Z, Klinger B. Effect of insulin-like growth factor-I treatment on serum androgens and testicular and penile size in males with Laron syndrome (primary growth hormone resistance). Eur J Endocrinol 1998;138:176-180.
39. Baker J, Hardy MP, Zhou J, Bondy C, Lupu F, Bellve AR, Eftratiadis A. Effects of a IGF-I gene null mutation on mouse reproduction. Mol Endocrinol 1996;10:903-918.
40. De Paepe ME, Waxman M. Testicular atrophy in AIDS: a study of 57 autopsy cases. Hum Pathol 1989;20:210-214.
41. Rappaport MS, Smith EP. Insulin-like growth factor-I inhibits aromatization induced by follicle-stimulating hormone in rat Sertoli cell culture. Biol Reprod 1996;54:446-452.
42. Prati M, Palmero S, De Marco P, Trucchi P, Fugassa E. Effect of insulin-like growth factor-I on Sertoli cell metabolism in the pubescent rats. Boll Soc Ital Biol Sper 1992;68:121-128.
43. Baxter RC. Insulin-like growth factor binding protein in the human circulation: a review. Horm Res 1994;42:140-144.
44. Guler HP, Zapf J, Schmid C, Froesch ER. Insulin-like growth factor-I and II in healthy man. Estimation of half-lives and production rates. Acta Endocrinol Copenh 1989;121:753-758.
45. Hodgkinson SC, Moore L, Napier JR, Davis SR, Bass JJ, Gluckman PD. Characterization of insulin-like growth factor binding proteins in ovine tissue fluids. J Endocrinol 1989;120:429-438.