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Insulinlike Growth Factor I in Combination With Insulinlike Growth Factor Binding Protein 3 Affects the Hepatic Acute Phase Response and Hepatic Morphology in Thermally Injured Rats

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Objective

To modulate the hepatic acute phase response after a thermal injury by the administration of insulinlike growth factor I (IGF-I) in combination with its principal binding protein 3 (IGFBP-3).

Summary Background Data

The hepatic acute phase response is a cascade of events initiated to restore homeostasis after trauma; however, a prolonged response contributes to multiorgan failure, hypermetabolism, complications, and death. Although IGF-1 has been shown to improve cell recovery and play a major role in liver regeneration, its effect on the hepatic acute phase response is not known.

Methods

Sprague-Dawley rats (56 males) received a 60% total body surface area third-degree scald burn and were randomly divided to receive either rhIGF-I/BP-3 (10 mg/kg/day given subcutaneously) or saline (control). Rats were killed on postburn days 1, 2, 5, and 7 and serum glucose, electrolytes, acute phase reactant proteins, tumor necrosis factor α, interleukin 1β, interleukin 6, and rat and human serum IGF-I and IGFBP-3 were measured. Hepatic protein concentrations, hepatocyte proliferation, and hepatocyte apoptosis were determined.

Results

No hypoglycemia or electrolyte imbalance could be shown in rats receiving the growth factor complex compared with saline. rhIGF-I/BP-3 increased serum protein on postburn days 2 and 7, albumin on days 5 and 7, and transferrin on days 1, 5, and 7, and decreased haptoglobin and α_1-acid glycoprotein on postburn days 5 and 7 compared with controls. IGF-I/BP-3 had no effect on type II acute phase proteins. Rats receiving IGF-I/BP-3 had lower serum levels of interleukin 1β and tumor necrosis factor α on the first day after burn compared with controls, whereas serum levels of interleukin 6 did not change. rhIGF-I/BP-3 significantly increased total liver protein content on postburn days 1, 2, 5, and 7 compared with controls. IGF-I/BP-3 increased hepatocyte proliferation and decreased hepatocyte apoptosis versus controls.

Conclusion

In combination with its principal binding protein, rhIGF-I decreases the proinflammatory cytokines interleukin 1β and tumor necrosis factor α, followed by a decrease in type I acute phase proteins. IGF-I/BP-3 had no effect on interleukin 6 and type II acute phase proteins. Decreases in acute phase protein and proinflammatory cytokine synthesis were associated with increases in constitutive hepatic proteins, total liver protein content, and hepatocyte proliferation. IGF-I/BP-3 attenuates the hypermetabolic response after thermal injury and may improve the clinical outcome.

The hepatic acute phase response represents a cascade of events characterized by the upregulation of type I and type II acute phase proteins and the downregulation of constitutive hepatic proteins.[1–5] These events are initiated to restore homeostasis after trauma.[1–7] Clinical studies, however, have shown that a sustained or increased acute phase response can contribute to multiorgan failure, hypermetabolism, complications, and death.[8–10] Syntheses of type I and type II acute phase proteins are mediated by proinflammatory cytokines; thus, the uncontrolled and prolonged action of proinflammatory cytokines is potentially dangerous.[8–10] In an attempt to attenuate the overexpression of proinflammatory cytokines and acute phase proteins, multiple clinical

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trials using inflammatory antagonists have been undertaken; however, these attempts have been unable to control the exaggerated hepatic acute phase response.[8,11,12]

Insulinlike growth factor-I (IGF-I) is a 7.7-kDa single-chain polypeptide of 70 amino acids with sequence homology to proinsulin.[13] Ninety-five percent to 99% of IGF-I is bound to one of six binding proteins (IGFBPs 1–6).[14] Administration of IGF-I has been shown to be an effective approach in the treatment of the trauma-induced hypermetabolic response by increasing peripheral muscle protein synthesis.[15] It improves cell recovery, wound healing, and gut and immune function after thermal injury[16–18] (unpublished observations). However, several adverse side effects such as hypoglycemia, electrolyte imbalance, edema, neuropathies, and cardiac arrest have limited its clinical application.[19,20] The cause of these side effects may be related to the supraphysiologic dose of free IGF-I required for effect.[19,20] Recently, a new form of IGF-I has been introduced in which IGF-I is bound to its principal binding protein, IGFBP-3.[21] This complex (IGF-I/BP-3) has been shown to be effective in hypermetabolic burned children without any detectable side effects.[21]

It has been suggested that overexpression of proinflammatory cytokines, such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α inhibit the anabolic growth hormone–IGF-I axis and contribute to increased rates of complications and death.[9,22–24] Growth hormone, an anabolic agent, has been shown to modulate the hepatic acute phase response.[25] Growth hormone, however, exerts its effects through IGF-I stimulation, which plays a major role in liver regeneration after injury.[15,20,26] In this study we determined the suitability of IGF-I/BP-3 as a therapeutic agent to attenuate the acute phase response and thus to improve clinical outcome after trauma. The efficacy of the complex was defined by examining the effects of IGF-I/BP-3 on constitutive hepatic proteins, acute phase proteins, proinflammatory cytokines, and hepatocyte proliferation and apoptosis.

MATERIALS AND METHODS

Sixty-four adult male Sprague-Dawley rats, weighing 300 to 350 g (Harlan-Sprague-Dawley, Houston, TX), were housed in wire-bottomed cages in a temperature-controlled room with a 12-hour light/dark cycle. Rats were acclimatized to the environment for 7 days. All received a liquid diet of Sustacal (Mead Johnson Nutritionals, Evansville, IN) and water ad libitum for the entire study period. All rats were pair-fed based on nutritional intake. The study was approved by the Animal Care and Use Committee of the University of Texas Medical Branch, Galveston, Texas, and followed the National Research Council's guidelines.

Fifty-six rats received a 60% total body surface area third-degree scald burn[27] under general anesthesia (pentobarbital 50 mg/kg) and analgesia (butorphanol 0.1 mg/kg) and were randomly divided into two groups to receive recombinant human IGF-I/BP-3 (10 mg/kg/day given intravenously; n = 28), or saline (0.4 mL/day given intravenously; n = 28). The dose of 10 mg/kg/day was determined in a dose-response study (unpublished observations). The rhIGF-I/BP-3 complex was provided by Celtrix Pharmaceuticals, Inc. (Santa Clara, CA) in a 1:1 molar ratio of rhIGF-I to rhIGFBP-3, corresponding to the naturally occurring protein complex, purified by cation exchange column chromatography. Injections were prepared from frozen vials containing 10.8 mL rhIGF-I/BP-3 (10 mg/mL) in sterile buffered solutions with 50 mmol/L sodium acetate and 105 mmol/L sodium chloride at pH 5.5. Treatment with rhIGF-I/BP-3 or saline was started 1 hour after burn injury to mimic the clinical setting. To determine side effects, rats were critically observed every day for behavior abnormalities, physical appearance, and differences in food intake. Body weights were measured daily.

Rats were killed by decapitation 1, 2, 5, or 7 days after injury. Serum was collected in serum separator tubes and plasma EDTA tubes, spun at 1,000*g* for 15 minutes, decanted, and frozen at −70°C until analysis. The entire liver was harvested, weighed, and sectioned. One section (500 mg) was fixed in 4% formalin for histologic examinations, one section (500 mg) was used for dry/wet ratios, and one (5 g) was snap-frozen in liquid nitrogen for storage.

Normal Levels

To establish normal values, eight rats without burn injury and without treatment received a liquid diet of Sustacal and water ad libitum for the entire study period. Animals were killed and tissue was taken as described above on days 1, 2, 5, and 7. These rats served as a nonburned, nontreated control group.

Serum IGF-I and IGFBP-3

To determine whether the recombinant human complex was biologically active and caused a response, serum human and endogenous rat IGF-I concentrations were determined using a human IGF-I radioimmunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA) or a rat IGF-I radioimmunoassay (Diagnostic System Laboratories, Webster, TX). Serum human BP-3 was determined using a human IGFBP-3 radioimmunoassay (Nichols Institute Diagnostics).

Serum Glucose, Electrolytes, and Constitutive and Acute Phase Proteins

Serum glucose, electrolytes, constitutive hepatic proteins (albumin and transferrin), and acute phase proteins (haptoglobin and α_2-macroglobulin) were measured using a Behring nephelometer (Behring, Dearfield, IL). Serum α_1-acid glycoprotein levels were determined using an enzyme-linked immunosorbent assay (Wako Chemicals Inc., Rich-

mond, VA). Standard curves using rat α_1-acid glycoprotein were linear from 0 to 1,500 pg/mL on a logarithmic scale.

Serum Cytokines

Plasma TNF-α levels were determined with a rat-specific enzyme-linked immunosorbent assay (Endogen, Woburn, MA). Standard curves for quantification of rat TNF-α were linear from 0 to 833 pg/mL on a logarithmic scale. IL-1β levels were determined using an enzyme-linked immunosorbent assay (Biosource Int., Camarillo, CA). Standard curves for quantification of rat IL-1β were linear from 0 to 1,500 pg/mL on a logarithmic scale. Serum levels of IL-6 were determined by bioassay using B9 cells (mouse hybridoma line) in their log phase of growth and treated with increasing concentrations of serum. Cell proliferation in response to serum addition was measured spectrophotometrically as previously described.[28]

Liver Changes

Hepatic water content was determined by measuring wet/dry weight ratios. Liver protein concentration was determined by protein assay (Bio Rad, Hercules, CA).

Proliferation

Liver cell proliferation was determined by immunohistochemical staining for proliferative cell nuclear antigen (PCNA). PCNA stains proliferating cells during the G_1-M cycle. To determine proliferating cells, sections were deparaffinized, rehydrated, and treated with proteases and HCl to decrease background contamination. Nonspecific antigen binding sites were bound by incubating sections with goat serum, after which the sections were incubated with PCNA–horseradish peroxidase conjugate (SC-56) at a 1:50 dilution overnight at 4°C, and then washed with phosphate-buffered saline. Finally, the sections were treated with diaminobenzidine-hydrogen peroxidase for 3 to 6 minutes under microscopic control and counterstained with Mayer's hematoxylin.

PCNA-positive cells (stained red-brown) were counted on two sections from each animal. In each section, two masked observers selected four different sections for counting PCNA-positive cells. Proliferating cells were identified as those with a brown staining of the nucleus or cytoplasm. All hepatocytes within the field were counted, and proliferation was expressed as a percentage of proliferating cells per 100 hepatocytes. Values for all sections were averaged to calculate proliferation for the liver of each animal.

Apoptosis

We used the TUNEL (terminal deoxyuridine nick end labeling) immunohistochemical method (Apoptag, Oncogene, Baltimore, MD) for histologic identification of apoptotic cells in the liver. Formalin-fixed tissues were processed and embedded in paraffin. Sections of 4 μm, obtained at 40-to 50-μm intervals, were deparaffinized, rehydrated in graded alcohol, and washed in deionized water. Protein was digested using proteinase K (20 μL/mL in phosphate-buffered saline) to decrease background contamination. The sections were then incubated with freshly prepared terminal deoxyribonucleotidyl transferase (TdT) enzyme at 37°C for 2 hours. After the enzyme incubation, the slides were incubated with antidigoxigenin peroxidase at room temperature for 30 minutes. The sections were thoroughly washed and diaminobenzidine-hydrogen peroxidase was applied for color development for 3 to 6 minutes under microscopic control. Lastly, the sections were counterstained with Mayer's hematoxylin and mounted.

Two sections of each block of tissue were obtained at 40- to 50-μm intervals. In each section, one masked observer selected five fields in four different sections ($\sim$5,500 cells) for counting TUNEL-positive cells. Apoptotic cells were identified as those with a brown staining of the nucleus, or as apoptotic bodies, which are fragments of apoptotic cells engulfed by neighboring epithelial cells.

All hepatocytes within the field were counted, and apoptosis was expressed as a percentage of apoptotic cells per 1,000 hepatocytes. Values for all sections were averaged to calculate apoptosis for the liver of each animal.

Statistics

Statistical comparisons were made by analysis of variance, followed by Bonferroni's correction and Student *t* test. Data are expressed as means $\pm$ SEM. Significance was accepted at $P < .05$.

RESULTS

All animals survived the 60% burn, and there were no dropouts. No differences in death rate could be demonstrated between saline and rhIGF-I/BP-3 treatment. No side effects were observed for rats receiving the complex during the study period. Those receiving IGF-I/BP-3 had an increase in body weight on postburn days 2, 5, and 7 compared with the control group ($P < .05$; Table 1).

Serum IGF-I

Serum human IGF-I and human IGFBP-3 levels were increased from days 1 through 7 after the burn in the rhIGF-I/BP-3 group compared with controls ($P < .05$). Endogenous rat IGF-I levels were higher in the controls than in those receiving rhIGF-I/BP-3 ($P < .05$; Table 2). These findings indicate that the recombinant human complex is biologically active in rats.

Serum Glucose, Electrolytes, and Constitutive and Acute Phase Proteins

No serum hypoglycemia or electrolyte imbalance could be shown for rats receiving the complex when compared

Table 1. BODY AND LIVER WEIGHT AND HEPATIC WATER AND PROTEIN CONCENTRATION

Group	Postburn Day 1	2	5	7
Saline				
Change in body weight (%)	0.1 ± 0.4	2.0 ± 0.7	−1.0 ± 0.5	−2.0 ± 0.9
Liver weight (g)	13.5 ± 0.8	18.0 ± 0.5	14.3 ± 0.7	15.0 ± 0.4
Wet/dry weight ratio (%)	0.66 ± 0.01	0.71 ± 0.01	0.69 ± 0.01	0.67 ± 0.01
Hepatic protein (mg/mL)	0.90 ± 0.02	0.87 ± 0.03	0.88 ± 0.02	0.92 ± 0.01
rhIGF-I/BP-3				
Change in body weight (%)	0.5 ± 0.3	6.0 ± 0.3*	1.0 ± 0.7*	0.5 ± 0.9*
Liver weight (g)	14.1 ± 0.5	17.0 ± 0.3	14.1 ± 0.4	14.0 ± 0.3
Wet/dry weight ratio (%)	0.65 ± 0.01	0.66 ± 0.01*	0.66 ± 0.01*	0.65 ± 0.01*
Hepatic protein (mg/mL)	0.92 ± 0.02	0.97 ± 0.04*	0.98 ± 0.05*	0.99 ± 0.04*

Data presented as mean ± SEM.
* Significant difference between rhIGF-I/BP-3 and saline ($P < .05$).
Normal levels: change in body weight +1% per week; normal liver weight 14–16 g; wet/dry weight ratio: 0.62–0.64%; hepatic protein 0.97–1.04 mg/mL.

with controls. Serum albumin levels were increased on postburn days 5 and 7 (Fig. 1), and serum transferrin levels were increased on postburn days 1, 5, and 7 in the IGF-I/BP-3 group versus controls ($P < .05$).

Administration of IGF-I/BP-3 decreased levels of type I acute phase proteins haptoglobin and α_1-acid glycoprotein on postburn days 5 and 7 days compared with controls ($P < .05$; Fig. 2). No difference between groups could be found in serum levels of type II acute phase protein α_2-macroglobulin.

Table 2. SERUM HUMAN IGF-I, HUMAN IGFBP-3, AND RAT IGF-I CONCENTRATIONS

Group	Postburn Day 1	2	5	7
Saline				
hIGF-I (μg/mL)	3.41 ± 0.12	3.62 ± 0.06	3.24 ± 0.04	3.13 ± 0.08
hIGFBP-3 (μg/mL)	ND	ND	ND	ND
Rat IGF-1 (μg/mL)	10.4 ± 0.8	12.7 ± 0.4	12.6 ± 0.5	17.2 ± 0.4
RhIGF-I/BP-3				
hIGF-L (μg/mL)	35.4 ± 4.4*	44.4 ± 6.6*	45.5 ± 11.2*	35.4 ± 3.9*
hIGFBP-3 (μg/mL)	5.6 ± 1.7*	7.7 ± 1.8*	6.0 ± 1.9*	6.2 ± 1.7*
Rat IGF-I (μg/mL)	5.4 ± 0.7*	8.1 ± 0.9*	7.1 ± 0.9*	7.6 ± 1.5*

ND, not detected in serum.
Data presented as means ± SEM.
* Significant difference between rhIGF-I/BP-3 and saline ($P < .05$).
Normal levels; hIGF-I 3–5 μg/mL; hIGF-I/BP-3 ND; rat IGF-I 19–25 μg/mL.

Serum Cytokines

Administration of rhIGF-I/BP-3 decreased serum levels of TNF-α on the first postburn day compared with controls ($P < .05$; Fig. 3). The complex also decreased serum levels of IL-1β on postburn days 1 and 2 compared with controls ($P < .05$). No difference between groups could be shown for serum IL-6 levels.

Liver Changes

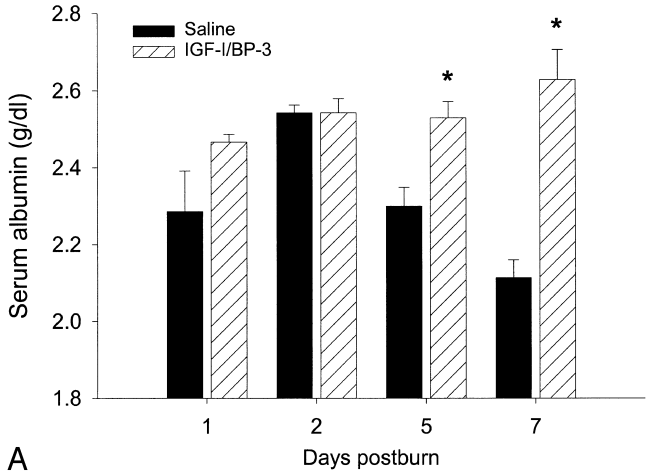
In both groups, liver weight increased on postburn day 2 compared with postburn days 1, 5, and 7. This increase in liver weight was associated with an increase in hepatic water content: the wet/dry weight ratio was increased in both groups 2 days after the burn compared with postburn days 1, 5, and 7. rhIGF-I/BP-3 treatment decreased the hepatic water assimilation on postburn days 2, 5, and 7 compared with controls. IGF-I/BP-3 significantly increased hepatic protein concentration on postburn days 2, 5, and 7 compared with controls ($P < .05$; Table 1).

Proliferation

Hepatocyte proliferation increased in both groups on postburn days 1, 2, and 5 when compared with physiologic hepatocyte proliferation. Rats receiving the IGF-I/BP-3 complex had increased mitogenic activity of hepatocytes on the first postburn day compared with controls ($P < .05$; Fig. 4).

Apoptosis

Hepatocyte apoptosis increased immediately after the burn in both groups and stayed elevated throughout the study period. rhIGF-I/BP-3 decreased hepatocyte apoptosis at postburn day 7 compared with controls ($P < .05$; Fig. 5).



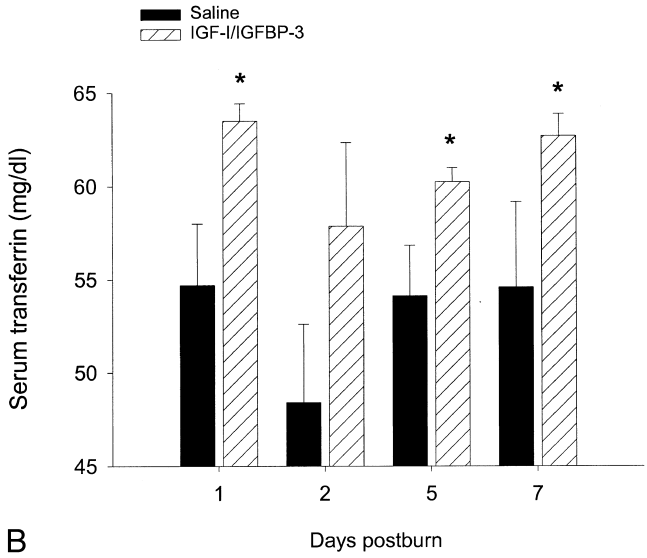
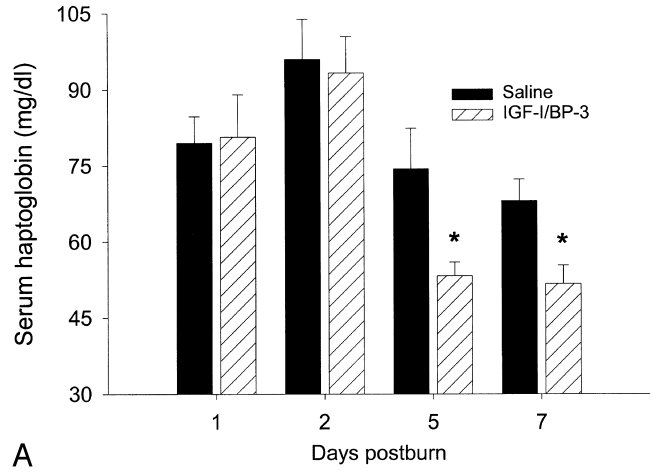


Figure 1. (A) Serum albumin levels decreased immediately after burn by nearly 40% below normal levels in both groups. IGF-I/BP-3 treatment increased serum albumin levels by postburn days 5 and 7 versus controls (*$P < .05$ vs. controls). Data presented are means ± SEM. Normal serum albumin level: 3.3–3.5 g/dL. (B) Serum transferrin levels decreased immediately after burn by nearly 80% below normal levels in both groups. Administration of rhIGF-I/BP-3 increased transferrin levels on postburn days 1, 5, and 7 versus controls (*$P < .05$ vs. controls). Data presented are means ± SEM. Normal serum transferrin level: >72 U/L.

DISCUSSION

The liver plays a critical role in the acute phase response after trauma.[1–5] To restore systemic homeostasis, the liver reprioritizes its synthesis from constitutive hepatic proteins toward acute phase proteins.[1–7] A prolonged increase in the acute phase response, however, has been shown to increase the rate of complications and death.[8–10] One possible cause for the increased rate is that proinflammatory cytokines, such as IL-1β and TNF-α, increase hypermetabolism and multiorgan failure.[9,10] A decrease in proinflammatory cytokine expression has been suggested to be beneficial after trauma.[10] Multiple clinical trials using antiinflammatory agents to attenuate the overexpression of proinflammatory cytokines have been described.[8,11,12] These agents, however, have failed to control the exaggerated synthesis of proinflammatory cytokines and acute phase proteins because they focused on only one pathway or mediator in the inflammatory cascade, leading to compensation through other pathways.[9,10] In the present study, we demonstrated that IGF-I decreased proinflammatory cytokines IL-1β and TNF-α, with subsequent decreases in type I acute phase proteins haptoglobin and α_1-acid glycoprotein, which all were upregulated after a thermal injury. Because we did not observe an increase in IL-6 or type II acute phase proteins, we propose that IGF-I can effectively decrease IL-1β and TNF-α levels without a compensatory elevation in IL-6 and type II acute phase proteins.

Another important contributor to the increased death rate



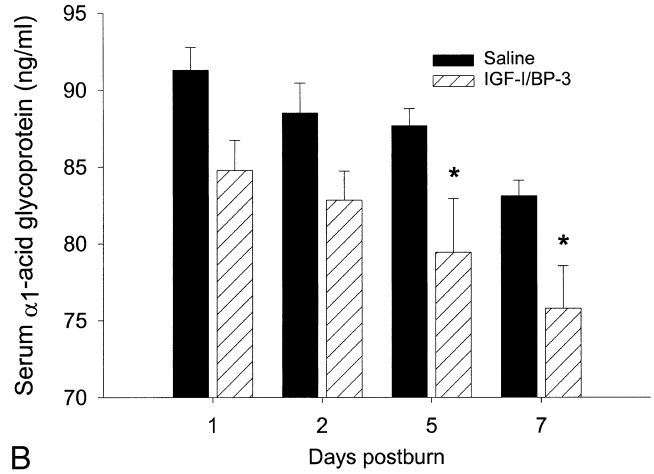


Figure 2. (A) Acute phase proteins increased two to three times above normal levels after burn injury. Administration of rhIGF-I/BP-3 decreased serum haptoglobin levels on postburn days 5 and 7 versus controls (*$P < .05$ vs. controls). Data presented are means ± SEM. Normal serum haptoglobin level: 5–32 U/L. (B) Administration of rhIGF-I/BP-3 decreased serum α_1-acid glycoprotein levels on postburn days 5 and 7 versus controls (*$P < .05$ vs. controls). Data presented are means ± SEM. Normal serum α_1-acid glycoprotein level: 5.5–14 ng/mL.

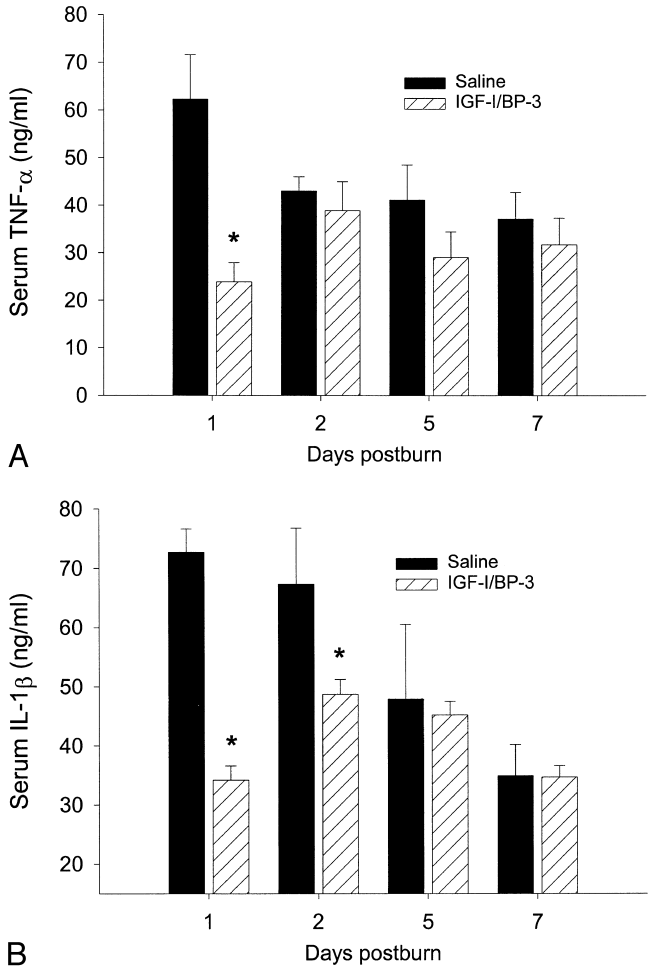


Figure 3. (A) Serum tumor necrosis factor α (TNF-α) levels after burn. Rats receiving IGF-I/BP-3 had significantly lower TNF-α level on the postburn day 1 versus controls (*$P < .05$ vs. controls). Data presented are means ± SEM. Normal serum TNF-α level: 1–10 pg/mL. (B) Interleukin (IL)-1β plasma concentrations after burn. IGF-I/BP-3 administration decreased IL-1β levels on postburn days 1 and 2 versus controls (*$P < .05$). Data presented are means ± SEM. Normal serum IL-1β level: 0.5–4 pg/mL.

is a decrease in the constitutive hepatic proteins after trauma.[9] After a burn, albumin and transferrin levels drop by 50% to 70% below normal due to the reprioritization of liver protein synthesis.[2–5] Albumin and transferrin, however, have important physiologic functions: they serve as transporter proteins and contribute to osmotic pressure and plasma pH.[2,3] Their downregulation after trauma has been described as potentially harmful, and the synthesis of these proteins has been used as a predictor of death, nutritional status, and severity of stress and as an indicator of improved recovery.[6,29–31] In the present study, we demonstrated that a burn decreased constitutive hepatic proteins. IGF-I/BP-3 increased serum albumin, transferrin, and total protein concentrations, all of which may be beneficial after a burn. We suggest that this increase in constitutive hepatic proteins may be due to a decrease in the production of acute phase proteins, which allows the liver at least in part to redirect its liver protein synthesis.

The signal pathway by which IGF-I modulates the hepatic acute phase response is not entirely defined. IGF-I may exert some of its effects through the transcription factors nuclear factor (NF)-κB and CCAAT/enhancer binding protein (C/EBP).[32–42] NF-κB controls the transcriptional regulation of many proinflammatory cytokines, including IL-1β and TNF-α.[32–35] Further, many type I acute phase proteins contain NF-κB response elements in their promoter region but not the type II acute phase proteins.[1,36,37] Modulating NF-κB activation may, therefore, cause a decrease in IL-1-like cytokines and type I acute phase proteins. The relative specificity of NF-κB for IL-1-like cytokines and type I acute phase proteins may explain why IGF-I had no effect on IL-6 and type II acute phase proteins. In addition, C/EBPβ is a transcription factor for acute phase proteins.[38,39] Growth hormone, which is known to increase IGF-I levels, has been shown to decrease C/EBPβ during the acute phase response.[40] Therefore, it can be surmised that IGF-I decreases C/EBPβ expression and subsequently the synthesis of acute phase proteins.

C/EBPα is a transcription factor for constitutive hepatic proteins such as albumin and transferrin.[38,39] The expression of C/EBPα has been shown to decrease after a burn.[38,39] Therefore, it seems likely that the decrease in constitutive hepatic proteins is associated with a decrease in C/EBPα expression.[38,39] IGF-I has been shown to increase C/EBPα expression; however, C/EBPα stimulates IGF-I.[41–43] We therefore speculate that IGF-I may exert some of its effects on constitutive hepatic proteins through stimulation of C/EBPα.

Preservation of organ homeostasis depends on a balance between cell proliferation and cell death.[44] Cell death can occur by two distinctly different mechanisms, apoptosis and necrosis. Apoptosis, or programmed cell death, is a genetically determined energy-dependent process by which senescent or dysfunctional cells are removed without extrusion of the intracellular contents or subsequent inflammation.[44] This is in direct contrast to necrosis, another mode of cell death, which is a passive process initiated by direct injury to the cell. A cutaneous thermal injury has been shown to induce small bowel epithelial cell apoptosis with a concomitant loss in cellular mass and the absorptive surface of the small bowel, and to induce apoptosis in myocardial cells with impairment in cardiac function.[45,46] In the present study, we have shown that a cutaneous burn induces hepatocyte apoptosis. Alterations in the balance between apoptosis and proliferation may lead to changes in organ function, integrity, and homeostasis.[47] Thus, it may be beneficial after a burn for the organ function either to increase proliferation or decrease apoptosis. In the present study, we demonstrated that IGF-I increased hepatocyte proliferation and liver protein synthesis, indicating that IGF-I attenuates the hypermetabolic response and diminishes the negative nitrogen balance. This is in agreement

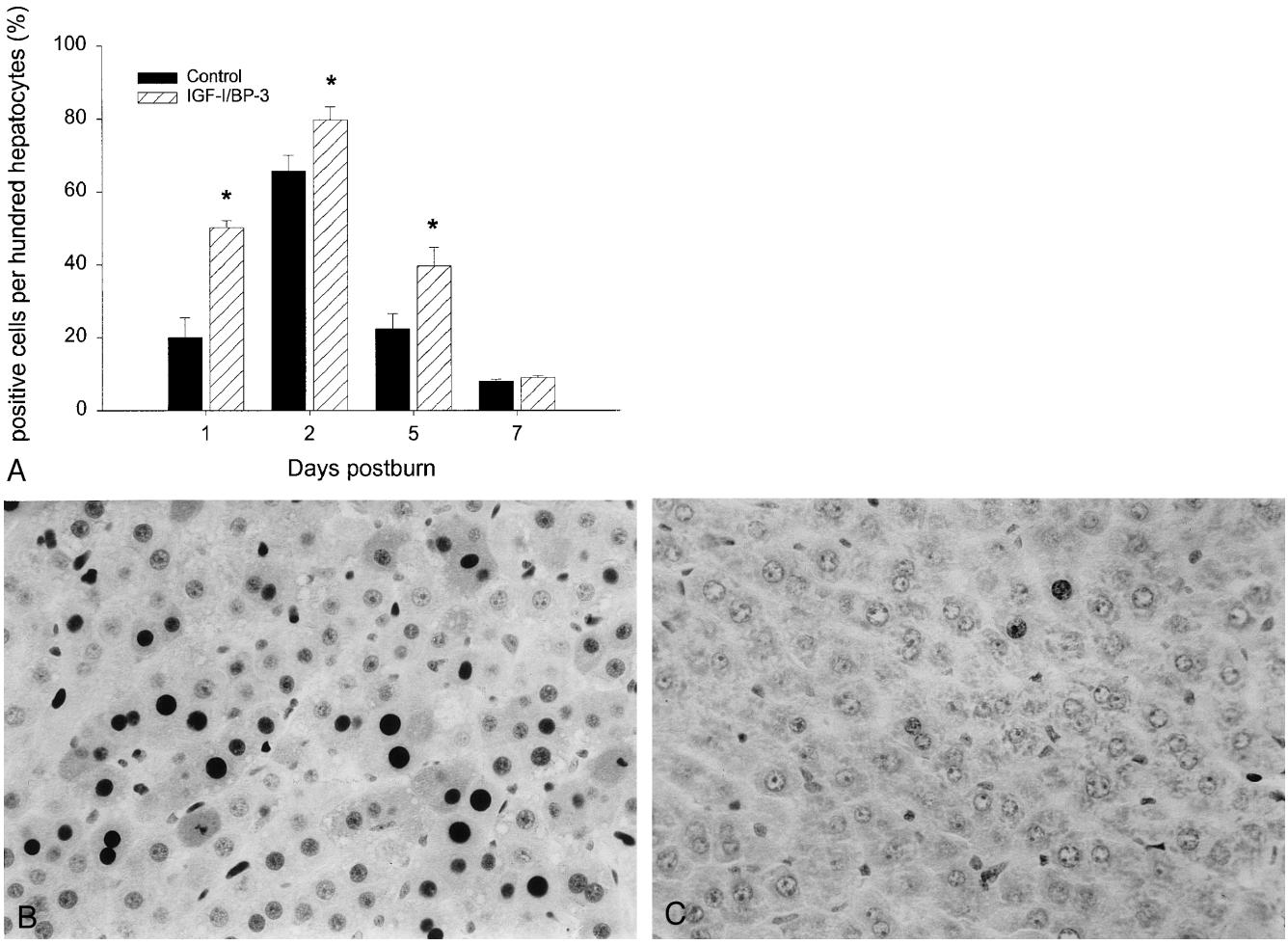


Figure 4. (A) Rats receiving rhIGF-I/BP-3 showed higher mitogenic activity of hepatocytes on postburn day 1 versus controls (*$P < .05$). Data presented are means ± SEM. Normal mitogenic activity: 1–5%. (B) Proliferation rate of hepatocytes (proliferative cell nuclear antigen [PCNA] index) in a representative section in burned rats receiving IGF-I/BP-3 for 1 day. Hepatocytes that stained dark brown were identified as hepatocytes that underwent mitosis and were considered to be positive. Magnification ×100. (C) Proliferation rate of hepatocytes (PCNA index) in a representative section in burned rats receiving saline for 1 day. Only a few hepatocytes were identified as having undergone mitosis (dark color). Magnification ×100.

with the results of other studies, where IGF-I has been shown to exert mitogenic activity, stimulate peripheral protein synthesis, and improve the metabolic rate and increase body weight.[15,16,48] We further demonstrated that IGF-I/BP-3 decreased hepatocyte apoptosis, which was found to be increased after a burn.

The mechanisms by which a cutaneous burn induces programmed cell death in hepatocytes are not defined. Studies have suggested that in general hypoperfusion, ischemia-reperfusion, and increased proinflammatory cytokines such as IL-1α/β, IL-6, and TNF-α are associated to promote apoptosis.[47,49] In the present study, we did not examine the effect of IGF-I/BP-3 on blood flow; however, we showed that IGF-I/BP-3 decreased the proinflammatory cytokines IL-1β and TNF-α. This attenuation may be the reason why IGF-I/BP-3 decreases hepatocyte apoptosis.

rhIGF-I in combination with its principal binding protein modulated the hepatic acute phase response by decreasing the proinflammatory cytokines IL-1β and TNF-α. This was followed by a decrease in the type I acute phase proteins. IGF-I/BP-3 had no effect on IL-6 and type II acute phase proteins. Increases in the constitutive hepatic proteins, total liver protein content, and hepatocyte proliferation were associated with decreases in acute phase protein and proinflammatory cytokine synthesis. However, using normal saline as a negative control does not allow differentiation between specific and nonspecific protein-mediated effects in the treatment arm of the study. Therefore, further studies using multiple groups with different growth factors are indicated to determine specific effects.

In summary, we suggest that attenuating the hepatic acute phase response after a burn with IGF-I/BP-3 modulates the hypermetabolic response and may prevent multiorgan failure, thus improving the clinical outcome in severe burns. Because we could not detect any side effects of IGF-I/BP-3 administration, we propose that the

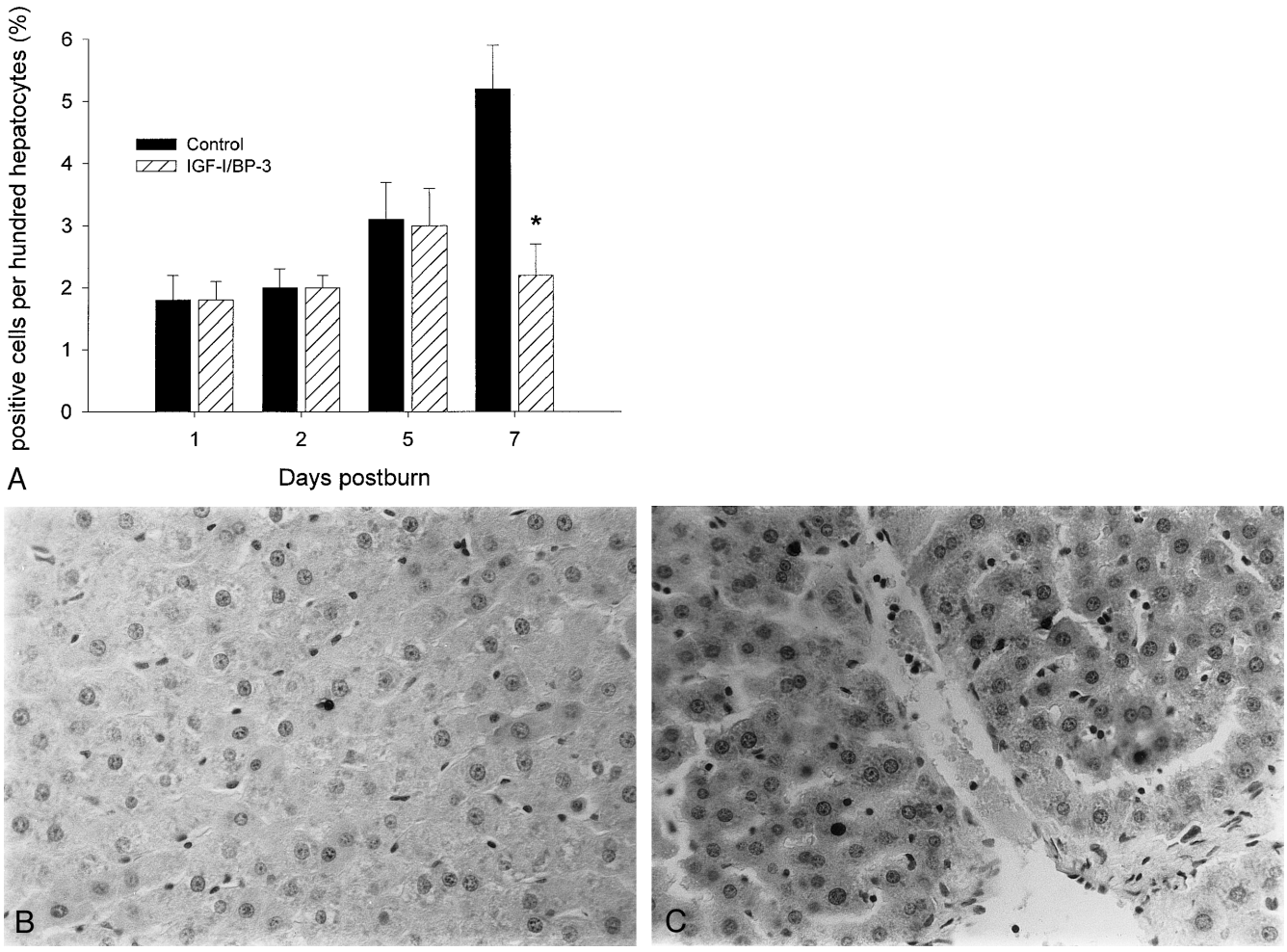


Figure 5. (A) Rats receiving saline showed higher apoptotic activity of hepatocytes on postburn day 5 versus treated rats (*$P < .05$). Data presented are means ± SEM. Normal hepatocyte apoptotic rate: 0.3 ± 0.1%. (B) Apoptotic rate of hepatocytes (TUNEL index) in a representative section in burned rats receiving IGF-I/BP-3. Only a few hepatocytes were identified that underwent apoptosis (stained dark brown) and were considered to be positive. Magnification ×100. (C) Apoptotic rate of hepatocytes (TUNEL index) in a representative section in burned rats receiving saline. Compared with B, more hepatocytes were identified as hepatocytes that underwent mitosis (dark color). Magnification ×100.

complex of IGF-I/BP-3 is a safe and efficient delivery system for IGF-I and may improve hepatic function after a cutaneous burn.[21]

An anabolic agent that has been used to improve the hypermetabolic response and the clinical outcome after a burn is recombinant human growth hormone (rhGH).[15,20,26] The administration of rhGH to burn victims has been shown to be beneficial by improving the hypermetabolic response, immune status, and wound healing.[15,20,26] However, recently it has been shown that rhGH administration increases the death rate in adult trauma patients, limiting its clinical use.[50] In light of the new data of Takala et al,[50] IGF-I/BP-3 may represent a new therapeutic approach to improve the survival rate after a burn or other form of trauma without side effects. We suggest that the complex may be clinically applied to severely burned patients or those suffering other forms of trauma.

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