Endogenous glucocorticoids released during acute toxic liver injury enhance hepatic IL-10 synthesis and release

MARK G. SWAIN, CAROLINE APPELEYARD, JOHN WALLACE, HOWARD WONG, AND TAI LE
Liver Unit, Gastroenterology Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1

Swain, Mark G., Caroline Appleyard, John Wallace, Howard Wong, and Tai Le. Endogenous glucocorticoids released during acute toxic liver injury enhance hepatic IL-10 synthesis and release. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G199–G205, 1999.—Endogenous glucocorticoids are known to play a role in the regulation of the inflammatory response possibly by modulating pro- and anti-inflammatory cytokine expression. We examined endogenous glucocorticoid secretion, hepatic damage, tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10) mRNA expression and release in rats treated with carbon tetrachloride (CCl4) after treatment with vehicle or a glucocorticoid receptor antagonist (RU-486). Rats treated with CCl4 demonstrated striking elevations of plasma corticosterone levels. Inhibition of endogenous glucocorticoid activity by pretreatment with the glucocorticoid receptor antagonist RU-486 resulted in augmented CCl4-inhibition on CCl4-induced hepatotoxicity, rats were treated from their cages, and truncal blood was collected. For plasma corticosterone level determinations, hepatic damage, tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10) mRNA expression and release. In summary, significant endogenous glucocorticoid release occurs during acute toxic liver injury in the rat and suppresses the inflammatory response independent of effects on TNF-α but possibly by upregulating hepatic IL-10 production.

METHODS

Animal model. Male Sprague-Dawley rats weighing ~200 g were obtained from Charles River (Pointe Claire, QC, Canada). All animals were housed in a light-controlled room maintained at 22°C with a 12:12-h day-night cycle and were given free access to food and water. The rats were handled regularly to avoid handling stress during experiments. All animals were treated humanely under University of Calgary Animal Care Committee guidelines.

The model of acute liver injury used was that due to CCl4 treatment (35). Rats were fasted for 12 h before and for 6 h after CCl4 (Sigma Chemical, St. Louis, MO) treatment. CCl4 (0.3 ml/200 g body wt in corn oil 1:1) or corn oil vehicle were given by gavage, rats were killed 0, 12, and 24 h later, blood samples were obtained by vena caval puncture (under halothane anesthesia) for TNF-α and transaminase level (ALT) determination, and liver samples were removed for histological examination. For plasma corticosterone level determinations rats were killed by decapitation within 30 s of removal from their cages, and truncal blood was collected.

To examine the effects of endogenous glucocorticoid activity inhibition on CCl4-induced hepatotoxicity, rats were treated with vehicle (0.2 ml of 0.9% saline) or RU-486, a glucocorticoid receptor antagonist (15) kindly provided by Roussel UCLA (10 mg/kg body wt in 0.9% saline). Vehicle or RU-486 was administered intraperitoneally every 12 h, starting 12 h before CCl4 gavage (28).

Serum transaminase and corticosterone determinations. Plasma alanine aminotransferase (ALT) levels were determined using a commercially available kit (Sigma). Plasma carbon tetrachloride (CCl4) is a hepatotoxic compound that is used widely as an inducer of acute and chronic experimental liver disease (35). Single-dose treatment of rats with CCl4 induces acute liver injury characterized by zone 3 liver cell necrosis and steatosis (35). TNF-α has been suggested as playing a role in liver cell damage due to CCl4 (9), as well as playing a central role in recovery from CCl4 hepatotoxicity (1, 3, 4).

Therefore, endogenous glucocorticoid secretion has been shown to be capable of modulating TNF-α synthesis and release in endotoxin-treated rats and mice (23, 36). Alternatively, administration of pharmacological doses of glucocorticoid appears to be able to increase the endogenous release of the anti-inflammatory cytokine IL-10 in humans and mice (19, 30, 32). Moreover, exogenous administration of IL-10 has been shown to protect the liver from toxic damage induced by a number of hepatic insults (17, 18).

Therefore in this series of experiments we quantified the magnitude of endogenous glucocorticoid secretion in rats treated acutely with CCl4 and examined the effects of the inhibition of endogenous glucocorticoid activity on the extent of CCl4-induced hepatotoxicity as well as on TNF-α and IL-10 synthesis and release.

INFLAMMATORY PROCESSES are capable of stimulating endogenous glucocorticoid secretion by activating the hypothalamic-pituitary-adrenal axis (13). This activation has been postulated to occur via proinflammatory cytokines [e.g., interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNF-α)] that act to stimulate all levels of the hypothalamic-pituitary-adrenal axis (13). Glucocorticoids (corticosterone in the rat) released from the adrenal cortex during the inflammatory process are known to play a role in the regulation of the inflammatory cytokine IL-10 in humans and mice (19, 30, 32). Moreover, exogenous administration of IL-10 has been shown to protect the liver from toxic damage induced by a number of hepatic insults (17, 18).

In summary, significant endogenous glucocorticoid release occurs during acute toxic liver injury in the rat and suppresses the inflammatory response independent of effects on TNF-α but possibly by upregulating hepatic IL-10 production.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

0193-1857/99 $5.00 Copyright © 1999 the American Physiological Society G199
Glucocorticoids in Hepatitis

Total RNA was extracted from incubating 1 µg of total RNA, 1 μl of random hexamer oligodeoxynucleotides (Pharmacia), 160 units of Superscript-reverse transcriptase (GIBCO-BRL, Burlington, ON, Canada), and 100 pmol of each deoxynucleotide triphosphates (dATP, dGTP, dCTP, and dTTP), 20 units placental ribonuclease inhibitor (RNAguard, Pharmacia), 160 units of Superscript-reverse transcriptase (GIBCO-BRL, Burlington, ON, Canada), and 100 pmol of random hexamer oligodeoxynucleotides (Pharmacia). Reaction mixtures were preincubated 10 min at 21°C before cDNA synthesis. The reverse transcription reactions were carried out for 50 min at 42°C and were heated to 95°C for 5 min to terminate the reaction. Reactions were performed in an Ampliton I thermal cycler (Barnstead/Thermolyne, Dubuque, IA).

Multiplex PCR reactions were performed using the primer dropping method as described by Wong et al. (34) in 50 μl reaction volumes containing 2 μl of RT reaction product as template DNA, 1× PCR buffer, 80 μM of each deoxynucleotide, and 20 pmol of each specific 5’ and 3’ primer. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA was amplified as an internal control for TNF-α and RPL 19 cDNA for IL-10. Two units of Taq DNA polymerase (Pharmacia) were added to each tube during the first denaturation step, and equal aliquots of secondary primer sets (20 pmol GAPDH or RPL 19) were added to the appropriate cycle number by the primer dropping method of Wong et al. (34). Briefly, cDNA was generated by a reverse transcription reaction by incubating 1 μg of total RNA, 1× PCR buffer (in mM, 10 Tris-HCl, pH 9.0, 50 KCl, and 1.5 MgCl2), 1 nM each of forward and reverse primers, 8 pmol of random hexamer oligodeoxynucleotides (Pharmacia). Reaction mixtures were preincubated 10 min at 21°C before cDNA synthesis. The reverse transcription reactions were carried out for 50 min at 42°C and were heated to 95°C for 5 min to terminate the reaction. Reactions were performed in an Ampliton I thermal cycler (Barnstead/Thermolyne, Dubuque, IA).

RESULTS

CCl4-induced corticosterone secretion. Plasma corticosterone levels were similar in oil-gavaged control rats 12 and 24 h after gavage (Fig. 1). However, CCl4 treatment resulted in striking elevations in plasma corticosterone levels at all time points after gavage compared with control levels at similar time points (Fig. 1).

RU-486 treatment and CCl4-induced hepatotoxicity. Serum ALT levels were significantly elevated 24 h after CCl4 treatment in vehicle- and RU-486-treated rats compared with basal levels (Fig. 2). However, serum ALT levels were strikingly higher in RU-486-
treated rats compared with vehicle-treated rats 24 h after CCl4 gavage (Fig. 2).

Histological examination of liver specimens revealed a marked reduction in steatosis but enhanced liver cell necrosis in RU-486-treated compared with vehicle-treated CCl4-gavaged rats, which was most pronounced 24 h after CCl4 administration (Fig. 3).

Glucocorticoid secretion and survival after CCl4. Adrenalectomized and sham adrenalectomized rats were gavaged with CCl4 and subsequently assessed 12 and 24 h later for survival. Throughout the 24-h observation period no CCl4-treated sham adrenalectomized rats died (n = 7). However, by 24 h after CCl4 administration five of seven adrenalectomized rats had died (P = 0.02). Administration of corticosterone to adrenalectomized CCl4-treated rats completely prevented CCl4-induced mortality in this group (n = 7).

Hepatic TNF-α mRNA expression and plasma TNF-α levels. The bioassay used in this study to detect circulating TNF-α is very sensitive, and serum levels of TNF-α in control rats similar to those detected in our experiments have been previously reported using this assay (10). Plasma TNF-α bioactivity increased significantly and to a similar degree 12 h after CCl4 gavage in vehicle- and RU-486-treated rats (Fig. 4). Plasma TNF-α bioactivity, however, had returned to levels similar to control rats by 24 h in both vehicle- and RU-486-treated animals (Fig. 4).

TNF-α mRNA expression was not detected in control livers. However, a striking increase in TNF-α mRNA expression was noted after CCl4 administration with levels peaking 12 h after CCl4 gavage in both vehicle- and RU-486-treated CCl4-gavaged rats (Fig. 5), consistent with previous reports (8). However, hepatic TNF-α mRNA expression was similar in vehicle- and RU-486-treated rats 12 and 24 h after CCl4 administration.
Hepatic IL-10 mRNA expression and plasma IL-10 levels. IL-10 is an anti-inflammatory cytokine that has recently been shown to be capable of protecting the liver from experimental hepatotoxicity (17, 18). Furthermore, recent evidence suggests that glucocorticoids can induce IL-10 synthesis and release (25, 30, 32). IL-10 was undetectable in plasma of control rats, consistent with observations of others (18). However, CCl₄ treatment resulted in a striking increase in plasma IL-10 levels by 12 h and this remained unchanged at 24 h post-CCl₄ (Fig. 6). Rats pretreated with RU-486 demonstrated a markedly attenuated increase in plasma IL-10 levels in response to CCl₄ 12 h after CCl₄ treatment (Fig. 6). By 24 h the plasma IL-10 levels in RU-486-pretreated CCl₄-gavaged rats were similar to levels observed in CCl₄-gavaged rats which were not pretreated with RU-486 (Fig. 6).

Low-level IL-10 mRNA expression was detectable in liver tissue from control rats. CCl₄ administration resulted in a 6.5-fold increase in hepatic IL-10 mRNA expression by 12 h, which was still evident 24 h after CCl₄ gavage (Figs. 7 and 8). However, CCl₄ administration in RU-486-treated rats resulted in no significant change in hepatic IL-10 mRNA expression compared with untreated controls (Figs. 7 and 8).
DISCUSSION

Acute hepatocellular necrosis induced by CCl4 is a widely used model of acute liver injury (35). We have shown that acute hepatic inflammation induced by CCl4 is a potent activator of endogenous glucocorticoid secretion and that enhanced glucocorticoid release plays a significant modulatory role with respect to the development of hepatic inflammation and overall survival after CCl4 treatment in rats. Furthermore, endogenous glucocorticoid release appears to be necessary for early release of the anti-inflammatory cytokine IL-10 in response to acute CCl4 hepatotoxicity.

The release of endogenous glucocorticoids from the adrenal gland during the inflammatory response plays a central role in the control of inflammation (reviewed in Ref. 6). However, the role played by endogenous glucocorticoid secretion in modulating hepatic inflammation and the mechanisms underlying this modulation are poorly documented. In the current study we have identified that a striking increase in plasma corticosterone levels accompanies the hepatic inflammatory response due to CCl4 administration. Moreover, the rise in plasma corticosterone levels precedes histological or biochemical indexes of CCl4-induced liver damage, suggesting that endogenous glucocorticoid secretion may modulate CCl4-induced hepatotoxicity. This hypothesis was confirmed by the demonstration of enhanced CCl4-mediated lethality in adrenalectomized rats (which is completely prevented by corticosterone treatment) and an augmentation of the biochemical and histological progression of CCl4-induced hepatotoxicity in rats where endogenous glucocorticoid receptors had been blocked by RU-486 treatment (RU-486 is a glucocorticoid receptor antagonist; 15). RU-486 is also a progesterone receptor antagonist (18). Given that progesterone can modulate inflammation by repressing NF-kappa B activity (reviewed in Ref. 31), some of the biological effects observed in our studies using RU-486 may possibly be due in part to effects of RU-486 on progesterone-mediated responses.

Early studies have suggested that endogenous glucocorticoids may exert some of their anti-inflammatory properties by inhibiting the production of TNF-α. Specifically, these studies demonstrated that adrenalectomized mice treated with endotoxin produced higher levels of TNF-α and were more susceptible to the lethal effects of endotoxin (23, 36). CCl4-induced hepatotoxicity is, at least in part, an endotoxin-mediated event (22). Furthermore, neutralization of TNF-α in rats treated with CCI4 has recently been shown to attenuate CCl4-induced hepatotoxicity (9). Therefore, our data demonstrating increased lethality in adrenalectomized rats treated with CCI4 compared with sham adrenalectomized controls would be consistent with these previous studies. However, we were unable to demonstrate enhanced hepatic TNF-α mRNA expression or plasma TNF-α levels in CCl4-treated rats in which endogenous glucocorticoid receptors had been blocked with RU-486 (as compared with CCI4-gavaged vehicle-treated controls). Our results with RU-486 are in agreement with previous studies in rats and mice that have shown that endogenous glucocorticoid blockade with RU-486 does not result in enhanced plasma TNF-α levels in response to endotoxin treatment (11, 24). These results suggest that TNF-α modulates CCl4-induced hepatotoxicity by direct or indirect effects that may be independent of specific serum or hepatic levels of this cytokine. Recently IL-10 has been demonstrated to be able to induce the production of soluble TNF-α receptors (16). Because soluble TNF-α receptors inhibit the biological activity of TNF-α, lower levels of hepatic IL-10 in RU-486-treated CCl4-gavaged rats may lead to the diminished local production of soluble TNF-α receptors within the liver, which could potentially result in augmented TNF-α-mediated hepatotoxicity in these animals.

Recent work has also suggested a possible beneficial role of TNF-α in the response of the liver to damage. TNF-α appears to play a critical role in hepatic repair after injury. TNF-α stimulates hepatic DNA and RNA synthesis and hepatic mitosis (3) and is of central importance in hepatic regeneration after partial hepatectomy in rats (1). Moreover, enhanced hepatic TNF-α levels have recently been shown to be necessary for the induction of early-immediate genes and in liver repair following CCl4-induced hepatotoxicity (4). The discrepancy between adrenalectomy and RU-486 effects on TNF-α release has been examined by Pettipher et al. (24) who found that elevated plasma TNF-α levels in endotoxin-treated mice are due to effects of released adrenal catecholamines, not glucocorticoids.

IL-10 is an anti-inflammatory cytokine, which plays a critical role in the natural defense against endotoxin-induced toxicity (2) and which has recently been shown to be capable of attenuating galactosamine-endotoxin and concanavalin-A-induced liver injury in mice (17, 18). The mechanisms underlying the hepatoprotective effects of IL-10 are poorly understood. IL-10 may attenuate CCl4-induced hepatotoxicity through its well-documented ability to decrease the production of many proinflammatory cytokines and chemokines (reviewed in Ref. 26). Furthermore, IL-10 can inhibit the recruitment of neutrophils to sites of inflammation by down-regulating the expression of endothelial cell adhesion molecules (14). Because neutrophils are known to contribute to CCl4-mediated hepatotoxicity (35), diminished neutrophil accumulation in the liver would be expected to attenuate CCl4-induced liver damage.
The liver appears to be the major source of circulating IL-10 (33). Moreover, glucocorticoids enhance IL-10 secretion from Th1 lymphocytes, and the administration of glucocorticoids to patients can increase serum IL-10 levels (25, 30, 32). Therefore, endogenous glucocorticoids may modulate inflammatory processes, at least in part, by enhancing IL-10 production. Our findings are consistent with this hypothesis. Endogenous IL-10 mRNA expression and IL-10 release were strikingly attenuated 12 h after CCl4 administration in rats receiving RU-486. By 24 h after CCl4 administration in RU-486-treated rats, plasma IL-10 levels were similar in RU-486 pretreated and nonpretreated groups, despite the observation that hepatic IL-10 mRNA levels remained low 24 h after CCl4 in RU-486-treated animals. Given that IL-10 has known hepatoprotective effects against hepatotoxins (17, 18) these data suggest that a relative lack of glucocorticoid-driven endogenous IL-10 secretion early in the course of acute CCl4 hepatotoxicity may predispose rats to more profound hepatic damage induced by hepatotoxins such as CCl4. The finding that at 24 h post-CCl4 treatment plasma IL-10 levels were similar in RU-486 pretreated and nonpretreated rats, despite a persistent decrease in hepatic IL-10 mRNA expression in the RU-486-pretreated rats, suggests that endogenous glucocorticoids have a complex modulatory role with respect to IL-10 production. Specifically, despite low hepatic mRNA levels in RU-486-pretreated rats 24 h after CCl4 gavage, IL-10 secretion appears to have normalized. However, given that serum transaminase levels and histological changes are significantly worse in CCl4 gavaged RU-486-pretreated rats compared with rats not treated with RU-486) 24 h after CCl4 treatment, the early hepatic IL-10 responses may be of critical importance with respect to hepatotoxicity evident at later time points.

In summary, we have demonstrated that acute liver necrosis due to CCl4 is associated with enhanced endogenous glucocorticoid secretion, which attenuates the early hepatic inflammatory response through a mechanism that appears to be independent of hepatic and serum TNF-α levels and possibly by enhancing local IL-10 production.

We thank Dr. Stefan Urbanski for assistance with the histological assessments.

M. G. Swain is an Alberta Heritage Clinical Investigator and a Medical Research Council (MRC) Scholar and J. Wallace is an Alberta Heritage Scientist and an MRC Senior Scientist. These studies were performed with an operating grant from the MRC of Canada to M. G. Swain.

Address for reprint requests: M. G. Swain, Liver Unit, Gastroenterology Research Group, Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1.

Received 17 March 1998; accepted in final form 1 October 1998.

REFERENCES


