Biochemical Pharmacology, Vol. 52, pp. 891–899, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00416-9

Effect of Chronic Alcohol Consumption by Rats on Tumor Necrosis Factor-α and Interleukin-6 Clearance *In Vivo* and by the Isolated, Perfused Liver

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ABSTRACT. The effects of chronic (16-week) alcohol consumption by rats on [^{125}I]tumor necrosis factor (TNF)-α and [^{125}I]interleukin (IL)-6 plasma clearance and organ distribution *in vivo* and uptake and metabolism by the isolated, perfused liver were studied. Alcohol was administered to rats in a liquid diet for 16 weeks, and caused a decreased (48%) plasma clearance rate of IL-6 and converted the plasma clearance kinetics of the cytokine from a biphasic exponential in normal rats to a monophasic exponential decay. Alcohol feeding significantly increased (101%) plasma clearance of TNF-α, which followed a biphasic exponential decay and decreased the $T_{1/2}$ for both the α (67%) and β (76%) elimination components. The distribution of both cytokines in trichloroacetic acid precipitable and non-precipitable fractions of liver, spleen, stomach, small intestine (ileum), lung, kidney, and blood was also studied. The only effect of alcohol treatment was a significant decrease in IL-6 uptake and metabolism by the small intestine. Perfused livers, isolated from alcohol-fed rats, took up and metabolized larger amounts of IL-6 than did livers isolated from pair-fed rats. TNF-α uptake and metabolism by the isolated, perfused liver were not affected by chronic alcohol consumption. Regardless of the animal treatment, the isolated perfused liver took up and metabolized significantly larger (17-fold) amounts of TNF-α than IL-6, in spite of identical concentrations of cytokines (6 nM) in the perfusion medium. The data presented in this study along with our previous results demonstrating the effects of alcohol consumption on TNF-α and IL-6 receptors on various liver cells suggest that the effects of chronic alcohol treatment on cytokine clearance cannot be ascribed to changes in the receptors for the two cytokines. Also, no correlation was found between the effects of alcohol treatment on plasma cytokine clearance and uptake and metabolism of cytokines by the isolated, perfused liver. Experimental data and theoretical considerations suggest that cytokine receptor recycling may play an important role in mediating alcohol effects on cytokine clearance. BIOCHEM PHARMACOL 52;6:891–899, 1996.

KEY WORDS. ethanol; liquid diet; radiolabeled cytokines; organ distribution; cytokine degradation

One of the major consequences of long-term alcohol abuse consists of a partial suppression of the immune defense processes [1–4]. Although the mechanisms underlying such suppression are not fully understood, it is widely accepted that alcohol interferes with the cytokine network. Such an interference affects various facets of the extraordinarily complex cytokine network, including cytokine synthesis and release by different cells (e.g. [5–9]), cytokine binding [7, 10–12], and the response of target cells to various cytokines [13–16].

Due to their involvement in almost every aspect of the immune function, the cytokines have been and are still under intense clinical and experimental scrutiny for their potential usefulness as therapeutic agents. Cytokine-based therapeutics comprise a number of strategies such as administration of cytokines, cytokine soluble receptors, and cytokine antagonists [17]. Within this context, it is obvious that pharmacokinetic studies of exogenously administered cytokines should precede any attempt to use cytokines as therapeutic agents. Moreover, whenever possible, such studies have to be performed under specific experimental conditions that would mimic as closely as possible clinical circumstances, such as chronic abuse of alcohol or other drugs, leading to partial alteration of various functions of the body.

Based on the above considerations, the purpose of this study was to determine if chronic alcohol consumption by rats affects the *in vivo* pharmacokinetics of two cytokines, TNF-α‡ and IL-6, and the uptake and degradation of these

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‡ *Abbreviations:* TNF-α, tumor necrosis factor-α; and IL-6, interleukin-6.

Received 23 January 1996; accepted 19 April 1996.

two cytokines by the isolated, perfused liver. The following reasons prompted to us to select these two cytokines. First, TNF-α has been the subject of investigation, both clinical and experimental, as a potential anticancer drug [18–22]. The other cytokine, IL-6, is known to control the acute phase response of the liver which plays an important role in the body's defense against inflammatory processes [23], and, as in the case of TNF-α, its secretion is also affected by alcohol [6]. Second, both cytokines are intensely secreted by the Kupffer cells, i.e. the liver resident macrophages, whose response to specific secretory stimuli, such as Gram-negative bacterial lipopolysaccharide, is known to be affected by chronic alcohol consumption [24, 25]. Hence, alcohol could alter cytokine pharmacokinetics, at least by inducing liver dysfunction. Third, earlier studies from our laboratory [10, 11] have shown that chronic alcohol consumption by the rat leads to alterations in the amount and affinity of receptors for both cytokines, on various liver cells, such as the hepatocyte, sinusoidal endothelial cell, and Kupffer cell. Therefore, it was of interest to determine if there is any link between alcohol-induced cytokine receptor alterations and the capacity of the liver to clear cytokines. Finally, one of the cytokines selected for this study, i.e. TNF-α, has been implicated as a causative factor in alcoholic liver disease [26].

The liver was selected for perfusion and cytokine clearance experiments because it is one of the organs most greatly affected by long-term alcohol abuse and, simultaneously, constitutes the main site of cytokine clearance from the blood [27, 28]. Therefore, it was of interest to assess a possible effect of chronic alcohol administration on the capacity of the liver to clear and metabolize the cytokines.

MATERIALS AND METHODS

The investigations reported in this study were conducted in conformity with principles outlined in *The Guide for the Care and Use of Laboratory Animals* (NIH Publication 85-23, revised 1985).

Animals and Their Treatment

CHRONIC ALCOHOL ADMINISTRATION. Male, Sprague–Dawley rats (Hilltop Laboratories, Scottdale, PA), weighing 75–100 g, were caged individually and started, 6 days after their arrival in the animal care facility, on a liquid diet, consisting of Sustacal (chocolate flavor, Mead Johnson Nutritional, Evansville, IN), supplemented with a mixture of vitamins (Dyets AIN-76A) and minerals (Dyets AIN-76), to which ethanol (6%, w/v, final concentration) or 8.4% (w/v) dextrin was added. The latter was calculated so as to provide the same amount of bioavailable energy as extracted from the alcohol, i.e. 36% of the total energy intake. The animals were weighed every 2 weeks, and the amount of food taken was monitored every day. At the end of the feeding period, which lasted 16 weeks, the body weight of the alcohol- and dextrin-fed rats was 391 ± 11 and 366 ± 9 g, respectively (N = 20 in each group, $P > 0.05$).

VASCULAR CATHETERIZATION. Twenty-four hours before the pharmacokinetic experiments, two PE50 catheters were implanted aseptically in the left jugular vein and right carotid artery and exteriorized on the dorsum of the animals. After surgery, the animals were returned to individual cages. On the day of the experiment, the catheters (approximately 20 cm long) were passed through a plastic sleeve (4 cm long, leaning on the dorsum of the animal), and affixed to the cage lid such that animals could move freely within the cage, while injection of cytokines (through the jugular catheter) or blood sampling (from the carotid catheter) was performed.

CYTOKINE ADMINISTRATION. Human recombinant [^{125}I]IL-6, with a specific radioactivity of 1400–3200 Ci · mmol^{-1}, and human recombinant [^{125}I]TNF-α, with a specific radioactivity of 600–800 Ci · mmol^{-1} (DuPont, NEN® Research Products, Boston, MA) were mixed with human recombinant IL-6 and TNF-α (Quality Controlled Biochemicals, Hopkinton, MA) and quickly injected into the jugular catheter in a volume of 0.30 mL · 100 g^{-1} body weight, corresponding to 10.4 μg · kg^{-1} body weight of IL-6 and 4.5 μg · kg^{-1} body weight of TNF-α, and 3.3 μCi · kg^{-1} body weight for both cytokines. After intravenous injection, the catheter was flushed immediately with 1 mL of sterile saline to avoid the retention of the cytokine within the PE50 tubing. The following reasons prompted us to select the cytokine doses employed in this study. First, they have been used by other investigators [29], thus making it possible to compare our data with those in other studies. Second, the selected doses induced cytokine concentrations in the plasma of the control animals within the range of 5–6 nM, which is higher than the K_D of the receptor–cytokine complex [10, 11]. Third, the initial plasma levels induced by these doses are within the range observed in the rat *in vivo* upon administration of stimuli such as Gram-negative bacterial lipopolysaccharide [30].

[^{14}C-METHYLATED]BOVINE SERUM ALBUMIN ADMINISTRATION. To determine the blood volume present in organ samples, dextrin- or alcohol-fed animals received intravenously 0.4 μCi · kg^{-1} body weight of [^{14}C-methylated] bovine serum albumin (DuPont, NEN® Research Products; 0.024 mCi · mmol^{-1}). The animals were killed 30 min after the tracer injection, and [^{14}C] radioactivity was determined as described below.

Blood and Tissue Sampling and Processing

Arterial blood (0.20 mL for each time point) was serially sampled with the aid of citrated syringes through the carotid catheter at time points shown in the corresponding figures. After each blood sample, 0.2 mL of sterile saline was injected into the carotid catheter. For the measurement of [^{125}I], blood (0.1 mL) was treated with 1 mL of trichloro-

acetic acid (20%, w/v), and centrifuged, and the supernatant was decanted into a glass tube. The pellet was washed once with trichloroacetic acid, and the supernatant was combined with the one resulting from the first centrifugation. The pellets and the supernatants were counted in a Beckman 8000 gamma counter (Beckman Instruments, Inc., Fullerton, CA). For the measurement of [^{14}C], blood (50 μL) was treated with 1 mL of tissue solubilizer (Beckman Tissue Solubilizer-450, Beckman Instruments, Inc.), decolorized with 0.2 mL of 30% H_2O_2, neutralized with 50 μL of glacial acetic acid, mixed with 10 mL of CytoScint (ICN, Costa Mesa, CA), and counted in a Beckman LS 1701 Scintillation Counter (Beckman Instruments, Inc.).

Plasma, obtained by centrifugation of blood at 1200 *g* for 10 min, was used for the assay of immunoreactive cytokines and ethanol (see below).

Samples (100–150 mg) of various organs (liver, spleen, stomach, small intestine (ileum), lung, and kidney) were collected 30 min after cytokine administration and processed for [^{125}I] determination as described above for the blood with the difference that organ samples were homogenized in an all-glass homogenizer in the presence of trichloroacetic acid. Tissue samples for [^{14}C] determination were treated with tissue solubilizer as described above. It is widely accepted that the [^{125}I] activity in the supernatant is a measure of metabolized cytokine, while the [^{125}I] in the pellet represents the amount of intact or partially degraded (still acid precipitable) cytokine.

Liver Perfusion

Livers were isolated from intact, non-catheterized animals and perfused as briefly described below. Under Nembutal® anesthesia (60 mg · kg^{-1} body weight, intraperitoneally), the abdominal cavity was opened, the portal vein was exposed and cannulated, and the liver was perfused at a flow rate of 3–4 mL · min^{-1}. The portal perfusate pressure was between 8 and 10 cm water. Immediately after cannulation and cutting the inferior vena cava (below the kidney), the flow rate was increased to 38–40 mL · min^{-1}. After cannulation of the supradiaphragmatic section of the inferior vena cava, the liver was perfused for 10 min in a flow-through system. After this preperfusion period, the perfusion system was switched to a recirculation mode. The perfusion medium during the 10-min preperfusion period was Krebs–Ringer bicarbonate buffer, continuously gassed with O_2:CO_2 (95%:5%) at 36.5°. During the recirculation period, the same buffer was used except that it contained 2% extensively dialyzed bovine serum albumin and [^{125}I]IL-6 or [^{125}I]TNF-α at a final concentration of 1 μCi and 6 nM (for both cytokines) in a final volume of 50 mL. The temperature and gassing conditions were identical to those during the preperfusion period. Perfusate samples (0.5 mL) were collected at the time intervals shown on the corresponding figures and used for radioactivity and immunoreactive cytokine assay.

The liver perfusion procedure employed in this study allows the organ to perform its basic metabolic functions, such as gluconeogenesis, ureogenesis, protein synthesis, and bile production [31, 32]. Scavenging of infused hyaluronan, which is taken as an index of the sinusoidal endothelial cell functional state [33], corresponds to what may be predicted on the basis of hyaluronan clearance *in vivo* [34]. Also, the scanning electron microscopic examination of the liver of dextrin-fed animals showed a normal appearance, as reported in the literature for livers of chow-fed rats [35].

Assay of Immunoreactive Cytokines and Ethanol

Appropriately diluted plasma and perfusate samples were used for both IL-6 and TNF-α assay (Biosource International, Camarillo, CA; Cytoscreen™ ELISA kits). Alcohol in the whole plasma was assayed with a commercially available kit (Sigma Chemical Co., St. Louis, MO; ethanol kit, Cat. No. 332A).

Data Processing and Statistics

The kinetics of cytokine clearance from the plasma and the liver perfusate, as well as the appearance of non-acid precipitable [^{125}I] in the perfusate were described using the GraphPad Prizm computer program (GraphPad, San Diego, CA). Area under the curve was calculated using the same program. The equations used to fit the data are mentioned in the legends to figures together with the correlation coefficient (r^2). The procedure described in Ref. [29] was used to calculate several pharmacokinetic parameters. The statistical significance of the difference was calculated using Student's *t*-test. A $P \leq 0.05$ was considered as indicating a statistically significant difference.

RESULTS

Characterization of the Chronic Alcohol Consumption Model Employed

The model of chronic alcohol consumption employed in this study, in which rats were fed for 16 weeks a dextrin- or alcohol-containing liquid diet, has been used in our laboratory for several years and has been characterized partially in earlier publications with regard to alcohol consumption, liver weight to body weight ratio, evolution of body weight, electron microscopic appearance of the liver sinusoid, and aspartate-2-oxoglutarate (EC 2.6.1.1) and alanine-2-oxoglutarate (EC 2.6.1.2) aminotransferase activity [35, 36]. The duration of feeding and the amount of alcohol administered were selected because earlier experimental data showed that under such conditions the liver sinusoid is structurally altered [35], and that liver pathology mimics some aspects of alcoholic liver disease in humans [36].

The alcohol levels in plasma at the time the animals were killed were 5.2 ± 0.5 mM for intact rats (used for liver perfusion experiments) and 6.2 ± 4.0 mM for catheterized rats (used for pharmacokinetic studies).

Plasma Levels of [^{14}C-methylated]bovine serum albumin in the rat

Measurement of [^{14}C-methylated]bovine serum albumin in the blood showed that the level of this compound was constant for at least 75 min after its administration, thus allowing the blood content of the organ sample to be estimated (not shown). The results of [^{14}C] measurements in blood and various organs were used to correct for the amount of blood present in organ samples in which [^{125}I] distribution was measured.

Kinetics of IL-6 and TNF-α in the Rat In Vivo

The main *in vivo* kinetic parameters of both cytokines in dextrin- and alcohol-fed rats are presented in Table 1 and Figs. 1 and 2. It is necessary to point out that these data were calculated on the basis of immunoreactive cytokine ELISA assays. A comparison between cytokine plasma levels calculated on the basis of immunoreactive assay or specific radioactivity of injected cytokine revealed no difference between the results obtained with the two calculation procedures. IL-6 clearance from plasma in dextrin-fed animals followed a biphasic exponential decay and displayed values for $T_{1/2}\alpha$ and $T_{1/2}\beta$ of 2.8 ± 0.01 and 614.9 ± 0.1 min. Alcohol feeding to rats changed the kinetics of IL-6 clearance to a monoexponential decay with a $T_{1/2}$ of 2.5 ± 0.5 min, which was associated with a decreased rate of clearance from 23.7 ± 2.1 mL · h^{-1} in dextrin-fed rats to 12.2 ± 0.4 mL · h^{-1} (Table 1).

As shown in Fig. 2, TNF-α clearance from plasma followed, as in the case with IL-6, a biphasic exponential decay without plateau. The half-lives for the fast and slow elimination components were 2.0 ± 0.1 and 20.9 ± 0.01 min, respectively. In alcohol-fed rats, the $T_{1/2}$ for both fast and slow elimination components was decreased by 67 and 77%, respectively. Also, the clearance rate was significantly higher (101%, Table 1) in alcohol-fed rats than in dextrin-fed animals.

A comparison of cytokine clearance in dextrin-fed animals with that observed in chow-fed rats, reported earlier by our laboratory [37], reveals that chronic feeding of the liquid diet of the type employed in this study leads to an overall decrease in cytokine clearance rate.

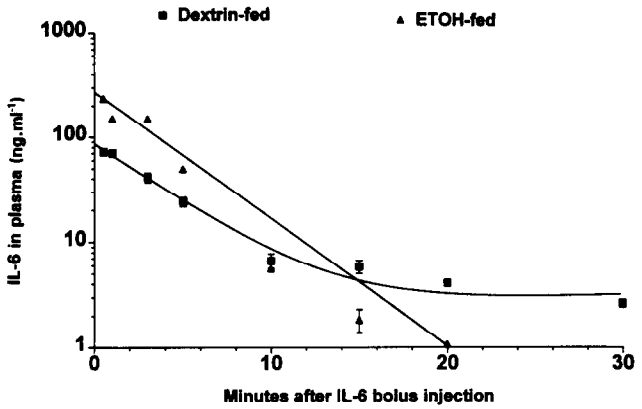

FIG. 1. Plasma clearance of human recombinant IL-6 in dextrin- and alcohol (ETOH)-fed rats. Plotted points are mean ± SEM (when not shown, the error bars were too small to be represented at the scale) for 4 animals in each group. The clearance followed a biphasic exponential decay for dextrin-fed rats and a monophasic exponential decay for ETOH-fed rats, with an r^2 of 0.999 and 0.979, respectively. Other pharmacokinetic details are given in Table 1.

Organ Distribution of IL-6 and TNF-α

As shown in Fig. 3 (pellet), 30 min after its administration, most of the non-metabolized (or partially degraded) IL-6 was concentrated by the kidney followed, in decreasing order, by the liver, blood, spleen, small intestine, lung and stomach. When calculated on the basis of the organ weight, it was obvious that the liver retained most of the cytokine (not shown). The only change induced by alcohol consisted of a significant decrease (82%) of non-metabolized (or partially degraded) IL-6 retention by the ileum (Fig. 3). A somewhat different picture was seen for the metabolized fraction of IL-6 (Fig. 3, supernatant). Thus, the small intestine contained the highest amount of non-precipitable [^{125}I], followed, in decreasing order, by the kidneys, liver, stomach, spleen, lung, and blood. Again, the only change induced by alcohol was a significant decrease (95%) in the acid-soluble [^{125}I] fraction in the ileum.

TABLE 1. Pharmacokinetic parameters of plasma IL-6 and TNF-α clearance in the rat

Experimental conditions	Parameters					
	Dose (ng · kg^{-1})	AUC (ng · min^{-1} · mL^{-1})	Cl (mL · hr^{-1})	Vc (mL)	$T_{1/2}\alpha$ (min)	$T_{1/2}\beta$ (min)
IL-6						
Dextrin-fed	10,380	436.8 ± 39.6	23.7 ± 2.1	125 ± 3.2	2.8 ± 0.01	614.9 ± 0.1
Ethanol-fed	10,380	848.8 ± 22.2	12.2 ± 0.4*	36 ± 0.2*	2.5 ± 0.5	
TNF-α						
Dextrin-fed	4,500	310.0 ± 36.8	14.5 ± 1.7	57.4 ± 0.1	2.02 ± 0.1	20.9 ± 0.01
Ethanol-fed	4,500	154.1 ± 15.4	29.2 ± 2.9*	58.2 ± 0.1	0.66 ± 0.1*	4.9 ± 0.01*

Values are means ± SEM, $N = 4$. Abbreviations and symbols: AUC, area under the curve; Cl, clearance; Vc, volume of distribution for the central compartment; $T_{1/2}$ = half-life time for the fast (α) and slow (β) component of clearance.

* Significantly different from the corresponding control (dextrin-fed group), $P < 0.05$.

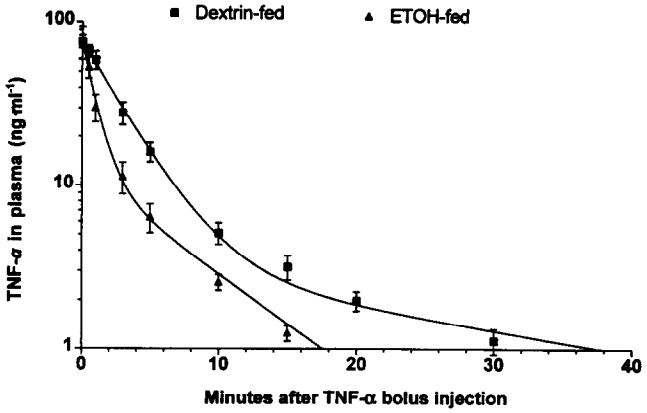

FIG. 2. Plasma clearance of human recombinant TNF-α in dextrin- and alcohol (ETOH)-fed rats. Plotted points are means ± SEM for 4 animals in each group. The clearance followed a biphasic exponential decay for both groups. The r^2 values were 0.909 and 0.830 for dextrin- and ETOH-fed groups, respectively.

The data depicted in Fig. 4 (pellet) show that at 30 min after TNF-α injection, most of the undegraded (or partially degraded) cytokine was present in the liver, followed, in decreasing order, by lung and kidney, spleen, blood, ileum,

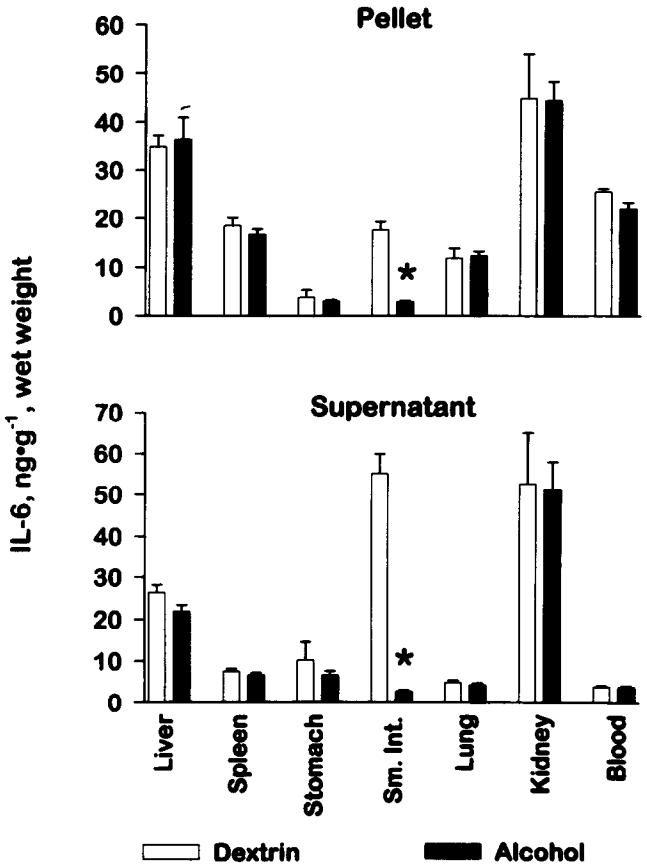

FIG. 3. Organ distribution of human recombinant [^{125}I]IL-6 at 30 min after its bolus injection. Values are means ± SEM for 4 animals in each group. The asterisk indicates a statistically significant difference between the groups at $P < 0.05$.

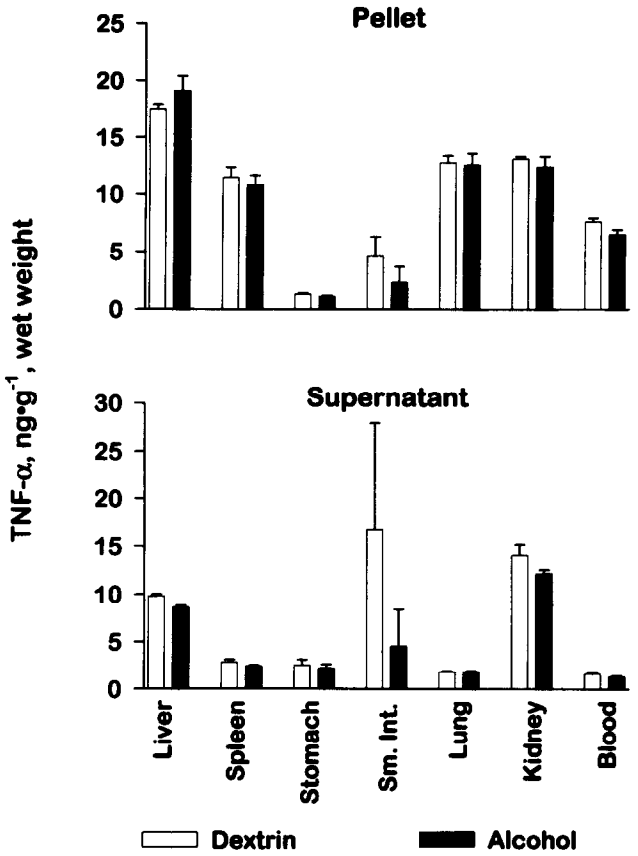

FIG. 4. Organ distribution of human recombinant [^{125}I]TNF-α at 30 min after its bolus injection. Values are means ± SEM for 4 animals in each group.

and stomach. No alcohol-induced alterations in cytokine organ distribution were observed. The fraction corresponding to metabolized TNF-α had a different organ distribution than the cytokine; thus, the highest level of non-precipitable [^{125}I] was found in the ileum, followed, in decreasing order, by the kidney, liver, with the spleen, stomach, lung and blood having the same level.

Cytokine Uptake and Degradation by the Isolated, Perfused Liver

The data from perfusion experiments were calculated on the basis of specific radioactivity of the cytokine presented to the liver. It is important to note that similar results were obtained when the uptake rate was calculated on the basis of the amount of immunoreactive cytokine, assayed in the perfusion medium with an ELISA kit, or on the basis of specific radioactivity of the cytokine presented to the liver.

The data depicted in Fig. 5 (upper panel) show that IL-6 uptake by the isolated, perfused rat liver of dextrin-fed animals was best described by a monoexponential association equation, with a virtual saturation attained toward the end of perfusion. While in livers of alcohol-fed animals the kinetics of IL-6 uptake were of the same type as in the livers of dextrin-fed rats, the rate of uptake was significantly

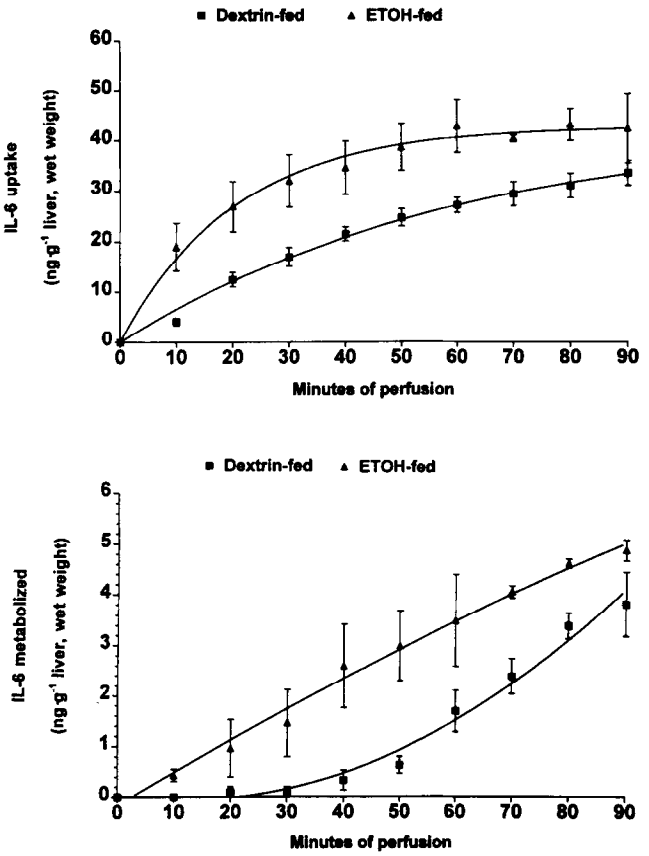

FIG. 5. Upper panel: Human recombinant [^{125}I]IL-6 uptake by the perfused liver, isolated from dextrin- or alcohol (ETOH)-fed rats. Plotted points are means ± SEM for 4 and 5 animals in dextrin- and ETOH-fed groups, respectively. The uptake followed saturation kinetics, described by a monoexponential association equation, with r^2 of 0.899 and 0.812 for the dextrin- and ETOH-fed groups, respectively. The difference between the groups was statistically significant at all time points up until 80 min of perfusion ($P < 0.05$). Lower panel: Human recombinant [^{125}I]IL-6 metabolism by the perfused liver, isolated from dextrin- or ETOH-fed rats. Plotted points are as for the upper panel. The results presented in the lower panel were obtained from the perfusions in the upper panel. The release of non-precipitable [^{125}I] products followed a linear regression for dextrin-fed rats and a second-order polynomial equation, with r^2 of 0.994 and 0.723 for dextrin- and ETOH-fed animals, respectively. The difference between the groups was statistically significant at all time points ($P < 0.05$).

higher, up until 80 min of perfusion, and reached a plateau at approximately 60 min after the start of perfusion. The rate of non-precipitable [^{125}I] appearance in the perfusion medium of the livers isolated from dextrin-fed rats (Fig. 5, lower panel) could be best described by a second-order polynomial equation with a lag of approximately 30 min. In livers isolated from alcohol-fed rats, the uptake rate was linear for the whole perfusion period and was significantly higher than in the livers of dextrin-fed rats (Fig. 5, lower panel). It is important to note that at the end of perfusion, the rate of IL-6 metabolism, judged on the basis of non-precipitable [^{125}I] appearance in the perfusion medium, ranged between 10 and 12% of the uptake rate at the plateau, with no difference between the treatment groups.

In comparison with the liver perfusions in which IL-6 kinetics were studied, the perfusion with TNF-α was performed for shorter periods of time (50 min), because the livers displayed a general leakage, which started around 50 min of perfusion. The kinetics of TNF-α uptake by the livers isolated from both groups were similar, being best described by a monoexponential association equation, with a plateau reached around 50 min of perfusion (Fig. 6, upper panel). No statistically significant difference was observed between the treatment groups, although in the alcohol-fed group a slight tendency toward a lower uptake rate could be noticed between 10 and 30 min of perfusion. The rate of appearance of non-precipitable [^{125}I] in the perfusion medium was linear as a function of time and almost identical for both treatment groups, reaching, at the end of perfusion, 20% of the uptake rate (Fig. 6, lower panel).

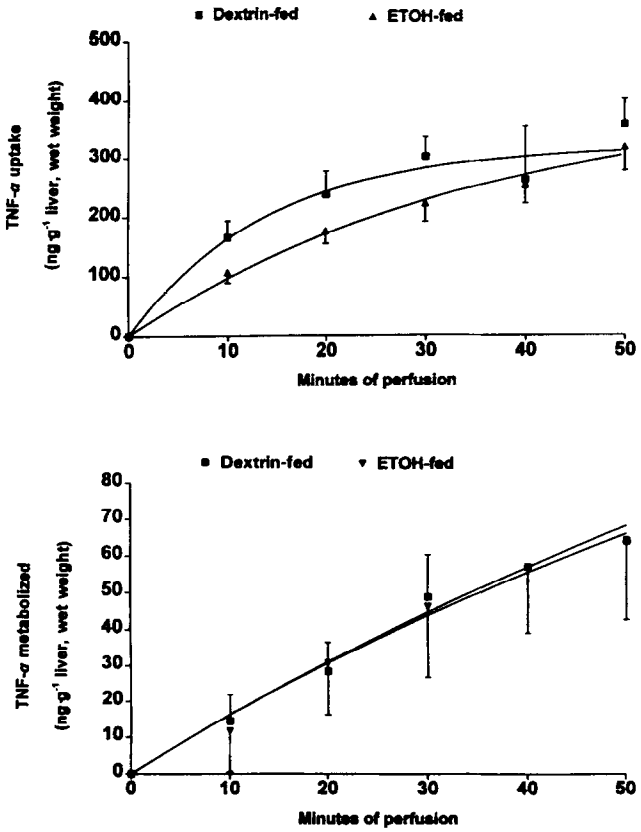

FIG. 6. Upper panel: Human recombinant [^{125}I]TNF-α uptake by the perfused liver, isolated from dextrin- or alcohol (ETOH)-fed rats. Plotted points are means ± SEM for 4 animals in each group. The uptake followed a saturation kinetics for both groups with r^2 of 0.848 and 0.816 for dextrin- and ETOH-fed rats, respectively, with no statistically significant difference between the groups. Lower panel: Human recombinant [^{125}I]TNF-α metabolism by the perfused liver, isolated from dextrin- or alcohol (ETOH)-fed rats. Plotted points are as in the upper panel. The results in the lower panel were obtained from the perfusion in the upper panel. The release followed linear regression with r^2 0.858 and 0.626, with no statistically significant difference between the groups.

A comparison of the uptake rates of the two cytokines revealed that, in spite of being present in the perfusion medium at the same final concentration, i.e. 6 nM, the uptake was much higher for TNF-α than for IL-6. Thus, at the same perfusion time, i.e. 50 min, the uptake was 25 $ng \cdot g^{-1}$ liver and 300 $ng \cdot g^{-1}$ liver for IL-6 and TNF-α, respectively, with a ratio of TNF-α/IL-6 of 12. The difference remains almost the same when the uptake is calculated on the basis of molecular weight of the cytokines (17,500 and 23,000 for TNF-α and IL-6, respectively, as given by the manufacturer), Thus, the above values for uptake become 1.0 and 17.1 $pmol \cdot g^{-1}$ liver, for IL-6 and TNF-α, respectively, with a ratio of TNF-α/IL-6 of 17. Essentially similar differences are seen in the non-precipitable [^{125}I] appearance in the perfusion medium.

DISCUSSION

Experimental data presented in this study allow an evaluation of the effects of chronic alcohol consumption by rats on IL-6 and TNF-α pharmacokinetics *in vivo* as well as on their uptake and metabolism by the isolated, perfused liver. The major effects of chronic alcohol consumption consisted of a decrease in the *in vivo* clearance of IL-6 and an increase in clearance of TNF-α, an enhancement of IL-6 uptake and metabolism by the isolated, perfused liver, and a diminution of the amount of IL-6 taken up and metabolized by the small intestine (ileum).

The first issue of interest raised by this study is a comparison of our data with those reported by other investigators [29, 38–40] with regard to the basic pharmacokinetic parameters for the two cytokines used. Studies performed with human recombinant IL-6 and TNF-α in the rat report a biphasic exponential decay for both cytokines, as it is in our study, and $T_{1/2}$ values for the fast (α) and slow (β) component of clearance very close to the ones presented in Table 1 in this study.

The major issue raised by the data reported herein pertains to the possible mechanism(s) by which alcohol affects cytokine clearance *in vivo* and by the isolated, perfused liver. The two primary mechanisms involved in cytokine clearance are: binding to specific receptors on different cells, including the blood-borne ones, followed by internalization and degradation, and excretion through the kidney. Since the latter aspect was not investigated in our study, we will limit our discussion to possible effects of alcohol on the first group of mechanisms.

The following analysis will show that no correlation can be demonstrated between the effects of chronic alcohol feeding on the following three parameters which define the fate of cytokines administered *in vivo* or to the perfused liver: IL-6 and TNF-α clearance from plasma (this study), IL-6 and TNF-α receptors on various liver cell types (earlier studies [10, 11], and the capacity of the liver to take up and to metabolize the cytokines (this study).

Receptors for IL-6 and TNF-α are located in many organs, most of which were investigated for the content of cytokines in our and other studies [41, 42]. It is currently accepted that the liver has a major quantitative contribution to the removal of the cytokines from the bloodstream [28, 29]. Therefore, one would expect that alterations in IL-6 and TNF-α receptor population on liver cells, induced by chronic alcohol feeding, might explain, at least in part, alcohol-induced changes in the rate of cytokine clearance. An analysis of alcohol-induced changes in IL-6 receptors of various liver cell types (the hepatocyte, sinusoidal endothelial cell, and Kupffer cell), under experimental conditions similar to the ones reported herein [10], reveals that chronic alcohol feeding to rats does not alter significantly the affinity (K_D) or the binding capacity (B_{max}) for human recombinant IL-6. Chronic alcohol feeding to rats was shown to augment significantly the TNF-α receptor population on the hepatocyte and sinusoidal endothelial cell, but not on the Kupffer cell [11]. At this point one should recall that while the clearance of IL-6 was diminished by alcohol, that of TNF-α was augmented by the drug. Taken together, these data do not support the assertion that the effects of alcohol on IL-6 and TNF-α receptors in the liver could account, at least in part, for the changes induced by alcohol in the rate of cytokine clearance. However, it is intriguing to note that chronic alcohol feeding to rats increased the capacity of the isolated liver to take up and metabolize IL-6 (Fig. 5). Moreover, such an increase was seen at an IL-6 concentration in the perfusate (6 nM) that was lower than in the plasma of alcohol-treated rats at zero-time (i.e. approximately 14 nM; Fig. 1), thus eliminating the concentration factor as contributing to the opposite effects of alcohol on the *in vivo* clearance and on the capacity of the liver to take up the cytokine.

When TNF-α data are analyzed, the lack of an alcohol effect on the capacity of the liver to take up and metabolize the cytokine did not appear to contribute to the alcohol-induced acceleration of TNF-α clearance *in vivo*. It should be pointed out that, as in the case of IL-6, the TNF-α level in the perfusate (6 nM) was close to the level in the plasma at zero time (4.4 nM, Fig. 2). Therefore, the concentration factor cannot explain the divergence between alcohol effects on the clearance *in vivo* and the lack of an effect in the perfused liver. An additional conclusion can be formulated on the basis of the above analysis: in spite of the fact that the liver is a major site for cytokine removal from the systemic circulation, significant changes in the organ cytokine receptors and in its capacity to take up IL-6 or TNF-α may not affect the cytokine clearance *in vivo*.

What then are the possible factors involved in alcohol-induced changes in cytokine clearance? On the basis of the experimental data presented in this study, two alternatives have to be considered: chronic alcohol consumption may induce alterations in the internalization of the cytokine–receptor complex and receptor recycling in various organs or chronic alcohol consumption may modulate the capacity of the kidney to eliminate the cytokines. With regard to the

first factor, recent experimental data from our laboratory show that chronic alcohol feeding to rats in a liquid diet did not affect the rate of TNF-α internalization.*However, the possibility that cytokine receptor recycling may be affected adversely by alcohol remains open. Clearly, more experimental work is needed to clarify this issue.

The data presented in this study also suggest that alcohol consumption may have important consequences on the kinetic behavior of endogenously secreted cytokines. It is known, for instance, that acute alcohol administration to rats *in vivo* suppresses the plasma TNF-α response to Gram-negative bacterial lipopolysaccharide [30]. It is not known, however, if this suppression is the result of the inhibition of cytokine secretion or the result of an accelerated clearance due to the presence of alcohol, as suggested by the results presented in this study for TNF-α.

Few studies have been published dealing with cytokine uptake and metabolism by the isolated, perfused liver. Among them, those by Pessina *et al.* [43] have shown that very little, if any, TNF-α is taken up by the isolated, perfused liver of rabbit and macaque (*Macacus mulata*) for perfusion periods of 200 min. This is somewhat intriguing in view of the data presented in our study, showing a substantial uptake of both cytokines by the isolated, perfused rat liver. Species differences may be one of the factors contributing to such a discrepancy.

It is interesting to note the significant difference between the uptake rates of IL-6 and TNF-α by the perfused rat liver. Despite the identical concentration of cytokines in the perfusate, i.e. 6 nM, the ratio of TNF-α to IL-6 uptake rate was 17 in control fed rats when calculated on the basis of the molecular weight of the cytokines. The rate of degradation of the two cytokines was also different with a ratio of TNF-α to IL-6 of 6.6 (Figs. 5 and 6). This is in agreement with the fact that, overall, the amount of TNF-α receptors of various liver cells exceed the amount of IL-6 receptors by a factor of 1.9 [10, 11]. However, besides the absolute amount of receptor sites, another factor, namely recycling of cytokine receptor, may be very important in determining the rate of uptake. Within this context, one has to recall that the time frame for cytokine receptor recycling has been found to be different for various cytokines [44–46]. One can also speculate that the difference between the effect of alcohol on IL-6 uptake and degradation and the lack of an effect of alcohol on TNF-α uptake and degradation by the isolated, perfused liver may be underlined by differential effects of alcohol on cytokine receptor recycling. This will have to be determined experimentally.

This study allows the following conclusions: (1) chronic alcohol consumption in the rat differentially alters plasma clearance of two cytokines, IL-6 and TNF-α; (2) alcohol induces changes in the uptake and metabolism of IL-6, but not of TNF-α, by the isolated, perfused liver; (3) such changes (in the case of IL-6), or the lack of change (in the case of TNF-α), are not consonant with alcohol-induced alterations in the receptors of either cytokine, observed in earlier studies; and (4) overall, chronic alcohol administration to rats had little effect on organ distribution of both cytokines, as assessed at one single time point, 30 min, after cytokine administration.

This study was supported by National Institute on Alcohol Abuse and Alcoholism Grant AA 09318.

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