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(Article begins on next page)

LIVER AP-1 ACTIVATION DUE TO CARBON TETRACHLORIDE IS POTENTIATED BY 1,2-DIBROMOETHANE BUT IS INHIBITED BY α -TOCOPHEROL OR GADOLINIUM CHLORIDE

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Abstract—Experimental acute intoxication by prooxidant haloalkanes produces marked stimulation of hepatic lipid peroxidation and cytolysis, which is followed by tissue regeneration. Our aim was to clarify the role of oxidative imbalance in the activation of the redox-sensitive transcription factor, activator protein-1 (AP-1), which is involved in tissue repair. Rats were poisoned with a very low concentration of carbon tetrachloride, given alone or in combination with another hepatotoxin, 1,2-dibromoethane, to provide varying extents of oxidative damage. The level of AP-1-DNA binding was analyzed by electrophoretic mobility shift assay on liver extracts, obtained from rats killed 6 h after poisoning. Stimulation of lipid peroxidation and AP-1 upregulation were already established when the hepatic damage due to carbon tetrachloride \pm 1,2-dibromoethane was beginning to appear. Rat supplementation with the antioxidant vitamin E completely inhibited AP-1 upregulation, thus supporting a causative role of membrane lipid oxidation in the observed modulation of the transcription factor. Moreover, activation of Kupffer cells appears to be a crucial step in the increased AP-1 binding to DNA, the latter being largely prevented by gadolinium chloride, a macrophage-specific inhibitor. © 1999 Elsevier Science Inc.

Keywords—AP-1, Carbon tetrachloride, 1,2-dibromoethane, Drug synergism, Macrophage, α -tocopherol, Free radical

INTRODUCTION

The prerequisite for tissue remodeling, such as that occurring after xenobiotic-induced cytolysis, is clearly the resetting of gene expression in the surviving cells. Cells regulate many steps in the pathway from DNA to proteins, but control of the initiation of transcription usually predominates. Transcription factors are proteins recognizing specific DNA sites in the regulatory regions of target genes; by interacting with RNA polymerase, they enhance or decrease its activity and transcription levels.

Most genes involved in cellular proliferation and differentiation [1,2] possess binding sites for activator protein-1 (AP-1) in their promoter region. AP-1 consists of

homo- or heterodimers of different polypeptides, such as Jun, Fos, or activating transcription factor (ATF) [1,3], and its activity has been demonstrated to be sensitive to reactive oxygen species (ROS) [4] as well as to lipid peroxidation end-products [5].

The experimental model of hepatic injury induced by carbon tetrachloride (CCl₄) has been used over the last few years to evaluate toxic interaction with 1,2-dibromoethane (DBE), another haloalkane widely polluting the environment, and also possessing marked necrogenic and carcinogenic properties. We have shown a strong synergism between these two drugs, in terms of enhanced lipid peroxidation and cytolysis, both in isolated hepatocytes [6] and in the whole rat [7]. Enhancement of AP-1-DNA binding has been reported in rat liver following acute administration of CCl₄ [8,9]. We deemed it of interest to investigate definitively whether modulation of AP-1 is actually correlated with the prooxidant action of haloalkane. In our model, rats were poisoned with a low dose

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of CCl_4 , given alone or in conjunction with DBE, to provide different extents of oxidative stress without the complications of marked cytotoxicity. The experimental plan included rat pretreatment with the antioxidant α -tocopherol, able to prevent the synergistic enhancement of lipid peroxidation by the two haloalkanes [10]. A final set of experiments was designed, using the macrophage-inhibitor gadolinium chloride (GdCl), to investigate the possible involvement of Kupffer cells in CCl_4 -dependent hepatic AP-1 upregulation.

Kupffer cells are macrophages present in the liver, anatomically juxtaposed, and functionally interdependent with hepatocytes [11,12]. Because Kupffer cells represent about 80–90% of the sessile macrophages of the reticuloendothelial system [13], and retain approximately 85–95% of the total intravascular phagocytic capacity [14], they represent the main site for clearance and detoxification of particulate and immunoreactive material [12]. Activated Kupffer cells play an important role in development of hepatotoxic injury caused by a number of xenobiotics [15–17], releasing chemo-attractants and activators of neutrophils, thus modulating the inflammatory response in the liver. Treatment with the selective inhibitor gadolinium chloride (GdCl) has been demonstrated to reduce liver injury in rats under ethanol or CCl_4 chronic treatment, possibly by reducing macrophagic secretion of inflammatory and cytotoxic products [17]. Whether Kupffer cells are also involved in the tissue remodeling observed after rat treatment with hepatotoxins is yet to be determined; the data reported here strongly suggest that they at least mediate the CCl_4 effect on AP-1 activity.

MATERIALS AND METHODS

Materials

All reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise specified.

Animals and treatments

Male Wistar rats (Harlan-Nossan, Correzzana, Italy), initial weight 200–220 g, were used in conformity with the ethical guidelines of the animal welfare committee of the University of Turin. They were randomly divided into experimental groups and fed with a standard pellet diet (Piccioni, Gessate Milanese, Italy) and water ad libitum. CCl_4 (125 $\mu\text{l}/\text{kg}$), DBE (20 $\mu\text{l}/\text{kg}$), or a mixture of CCl_4 and DBE were administered by gastric intubation to the experimental groups, as appropriate; control rats received mineral oil. Animals were killed 6 or 12 h after poisoning, by exsanguination under halothane anesthesia. Blood was collected and serum separated by

centrifugation, and stored at -80°C . The liver was perfused with saline solution, removed, frozen in liquid nitrogen, and stored at -80°C .

Alpha-tocopherol was dissolved in 1 volume of 95% ethanol, and 9 volumes of 16% Tween 80 in 0.9% NaCl were added to the solution, which was then injected i.p. as a single dose (100 mg of α -tocopherol/kg), 17 h before poisoning. Control rats received equivalent volumes of vehicle. Gadolinium chloride (Aldrich Chemical Co., Milwaukee, WI, USA) dissolved in saline solution, was injected into the tail vein in a single dose (10 mg/kg), 24 h before poisoning. The actual extent of the GdCl-induced inhibition of Kupffer cell phagocytic activity was checked by monitoring the colloid carbon clearance from blood [18] in GdCl + and GdCl- rats, treated or not treated with haloalkanes. This clearance test also afforded a morphological estimate of the different levels of macrophage activation in liver from control and intoxicated animals.

Morphological evaluation of carbon particle uptake

Liver fragments of about 0.5 cm, taken from the medial lobe of control and CCl_4 + DBE-treated rats, with or without previous treatment with GdCl, were placed in 4% formaldehyde buffered solution (pH 7.4) for 6 h at 4°C , washed in 0.1 M phosphate buffer (pH 7.4), then cryoprotected in 30% sucrose buffered solution (pH 7.4), and finally snap-frozen in liquid nitrogen and stored at -80°C . The uptake of carbon particles was evaluated in unstained 10- μm cryosections by light microscopy.

Biochemical analyses

The following enzymatic activities were evaluated for each group of 3–6 rats: sorbitol dehydrogenase (SDH) [19], glutamic-pyruvic-transaminase (GPT) [20] (kit from Boehringer Mannheim GmbH, Mannheim, Germany), and glutamic-oxaloacetic-transaminase (GOT) [21] (assay kit from Boehringer Mannheim GmbH).

Lipid peroxidation susceptibility was evaluated by measuring the level of thiobarbituric acid reacting substances (TBARS) [22] on liver homogenates (5% w/v), incubated in a shaking bath at 37°C for 2 h, from normal and 6 h-treated rats.

Preparation of liver extracts

The liver tissue from rats killed 6 h after poisoning was finely sliced with a scalpel blade, rinsed twice with ice-cold phosphate-buffered saline, and incubated for 1 h at 4°C in a high-salt detergent buffer, defined as Buffer

TOTEX, (20 mM HEPES [pH 7.9], 350 mM NaCl, 20% glycerol, 2% Nonidet-P40, 0.5 mM ethylenediaminetetraacetic acid, 1 mM MgCl₂, 0.1 mM ethylene-glycol-bis(β -aminoethylether)-N,N,N',N'tetraacetic acid, 10 μ g/ml leupeptin, 5 mM dithiothreitol, 1% aprotinin, 30 μ M phenylmethylsulfonylfluoride). The lysate was then centrifuged for 10 min at 20,000 $\times g$ at 4°C; the supernatant was diluted with a buffer containing 25 mM HEPES (pH 7.6), 20% glycerol, 0.1 mM ethylenediaminetetraacetic acid, 5 mM dithiothreitol, 30 μ M phenylmethylsulfonylfluoride, 1% aprotinin, and 10 μ g/ml leupeptin, then divided into 10- μ g aliquots and stored at -80°C. The protein content was measured as described by Bradford (Bio-Rad Laboratories GmbH, Munchen, Germany) [23].

Electrophoretic mobility shift assay

Binding assays were performed for 20 min at room temperature with 10 μ g of protein in 20 μ l of reaction mixture containing 20 μ g bovine serum albumin, 2 μ g poly (dI-dC) (Boehringer Mannheim GmbH), 2 μ l buffer D (20 mM HEPES [pH 7.9], 20% glycerol, 100 mM KCl, 0.5 mM ethylenediaminetetraacetic acid, 0.25% Nonidet-P40, 0.1% phenylmethylsulfonylfluoride, 2 mM dithiothreitol), 4 μ l buffer F (20% Ficoll-400, 100 mM Hepes [pH 7.9], 300 mM KCl, 10 mM dithiothreitol, 0.1% phenylmethylsulfonylfluoride), 5 mM MgCl₂ and 50,000 cpm of ³²P-labeled AP-1 oligonucleotide (Promega, Madison, WI, USA). DNA-protein complexes were separated from unbound DNA probe by electrophoresis on native 4% polyacrylamide gels at 200 V in 45 mM Tris-borate, pH 8.0, containing 1 mM of ethylenediaminetetraacetic acid. Radioactivity of dried gels was detected by exposure to Kodak XAR-5 film. Competition experiments were performed by incubating the extracts with the labeled probe, in the presence of 150-fold excess of unlabeled NF- κ B or AP-1 oligonucleotide. For antibody supershift assays, antisera recognizing the different members of the AP-1 family were preincubated with 5 μ g of extract for 90 min on ice (α JunB, α c-Fos, α FosB, and α FraII were obtained from Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; α c-Jun 1.4, α JunD 777.2, and α FraI were kindly provided by Prof. A. Azzi).

Statistical analyses

The significance of differences between the experimental groups was evaluated by Student's *t*-test and by one-way ANOVA test.

Table 1. Effect of CCl₄ and/or DBE on Cytolysis

Groups	SDH mU/ml	GPT mU/ml	GOT mU/ml
6-h treatment			
Control	3.48 \pm 0.54	20.9 \pm 9.87	58.3 \pm 7.58
CCl ₄	19.19 \pm 1.89*	40.7 \pm 18.69*	94.5 \pm 9.46*
DBE	4.22 \pm 0.47	21.0 \pm 8.56	58.2 \pm 10.30
CCl ₄ + DBE	21.42 \pm 2.52*	50.6 \pm 12.35*	122.2 \pm 17.80*
12-h treatment			
Control	3.50 \pm 0.61	16.8 \pm 2.08	51.9 \pm 6.43
CCl ₄	181.11 \pm 25.32*	82.9 \pm 17.19*	520.1 \pm 49.91*
DBE	4.04 \pm 0.22	17.9 \pm 2.13	71.2 \pm 2.85
CCl ₄ + DBE	164.53 \pm 5.51*	113.5 \pm 33.42*	708.4 \pm 52.28*

Data are means \pm SD of 3-6 rats per group.

*Significantly different from control group (*p* < .001).

RESULTS

Marked upregulation of AP-1 binding in the liver of CCl₄-treated rats; synergism between CCl₄ and DBE

Electrophoretic mobility shift assay was performed on hepatic extracts from animals treated with a single dose of CCl₄ alone, DBE alone, or CCl₄ + DBE, at 6 h into intoxication, when the cytolytic indexes SDH, GOT, and GPT (Table 1), were slightly above the normal range in the groups exposed to CCl₄ or to the mixture. CCl₄ treatment produced a strong induction of AP-1 binding, while DBE produced no activation of the transcription factor (Fig. 1). Moreover, when the two haloalkanes were administered simultaneously, a dramatic synergistic effect on AP-1 binding was consistently observed (Fig. 1). At 6 h, while tissue damage was just appearing (Table 1), the effect on lipid peroxidation exerted by CCl₄ and potentiated by DBE, measured in terms of TBARS production, was already expressed (Fig. 2).

Competition experiments, using either the AP-1 or the NF- κ B consensus sequences, confirmed that the DNA-protein complexes shown were specific for the AP-1 binding motif (Fig. 1). In addition, by means of specific antibodies against the main components of the AP-1 complex, the presence of c-Jun, JunB, and FraI in the gel-shifted bands was demonstrated (data not shown).

Lipid peroxidation is probably involved in the genesis of CCl₄ \pm DBE-induced AP-1 binding

Pretreatment with a single dose of vitamin E, able to prevent lipid peroxidation induced by CCl₄ (Fig. 2) [10], also afforded complete inhibition of the upregulation of AP-1 DNA binding due to the CCl₄ and CCl₄ + DBE (Fig. 3).

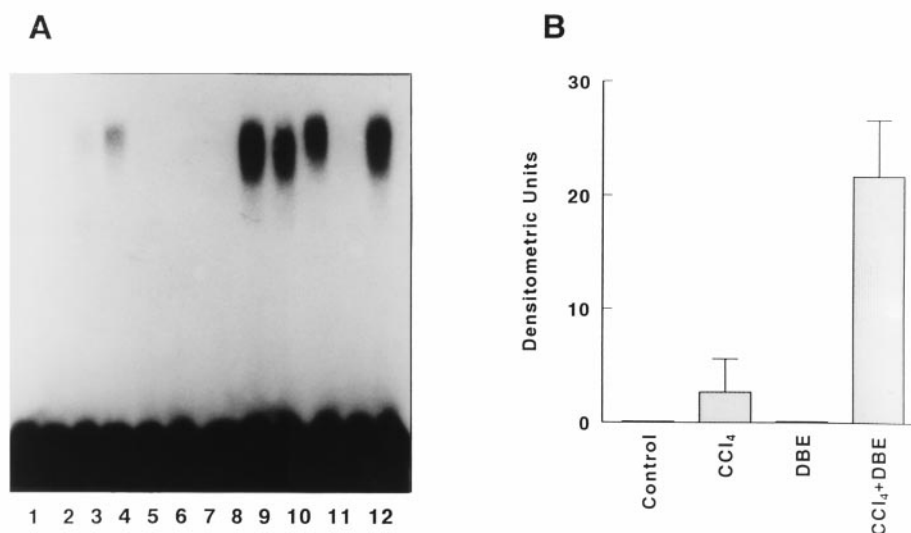


Fig. 1. Hepatic AP-1 binding activity: synergism between CCl₄ and DBE. (A) Liver extracts were incubated with radiolabeled AP-1 oligonucleotide, and protein-DNA complexes were separated from unbound DNA by electrophoresis. Lanes 1, 2: mineral oil; lanes 3, 4: 6 h after poisoning with CCl₄; lanes 5–7: 6 h after poisoning with DBE; lanes 8–10: CCl₄ + DBE. In lanes 11 and 12, specificity of the retarded complex was assessed by incubating a sample of lane 8 with a labeled probe, but in the presence of 150-fold excess of unlabeled AP-1 or of NF- κ B oligonucleotide, respectively. (B) Data are the mean changes in AP-1 density band \pm SD of four different animals. Density of bands was quantitated by Imaging Densitometer (Bio-Rad) and expressed in arbitrary densitometric units.

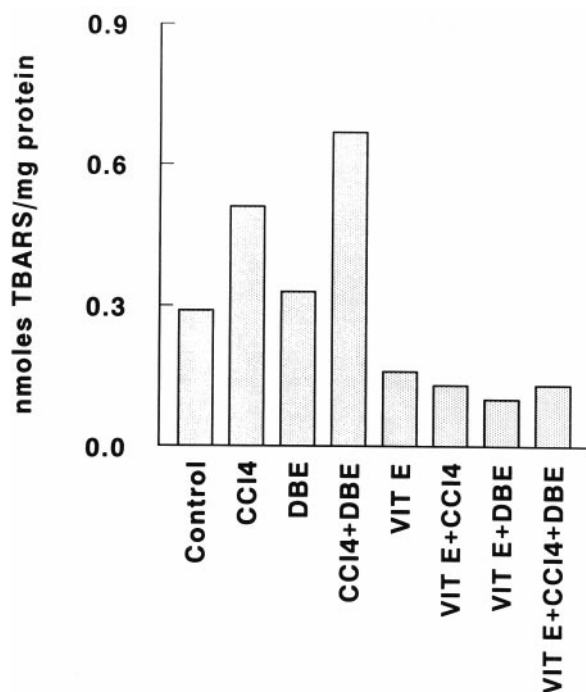


Fig. 2. Effect of poisoning with the haloalkanes on the susceptibility of the rat hepatic tissue to lipid peroxidation. TBARS production by liver homogenates obtained from control and treated rats after 6-h treatment, followed by 2-h incubation at 37°C. Data are means \pm SD of 2–4 different experiments.

Kupffer cells are the key players in AP-1 upregulation observed in hepatic tissue of rats challenged with CCl₄ \pm DBE

With the aim of determining presence and location of activated Kupffer cells in the liver intoxication model, the carbon clearance test and morphological observations were performed (see Methods section). As shown in Fig. 4, a specific rate of uptake of colloid carbon from the bloodstream was observed in the non-poisoned animals. Carbon clearance increased moderately in rats intoxicated with the haloalkanes (Fig. 4). Indeed, light microscopy gave a more direct and reliable answer than did the carbon clearance method; a very strong difference in carbon uptake between control and haloalkane treatment-derived liver slices was shown (Figs. 5A, 5B). The increase in phagocytic activity by Kupffer cells from poisoned livers was predominantly localized in the periportal zone of the hepatic lobule (Fig. 5B) and, interestingly, some carbon particles were also taken up by endothelial cells (Fig. 5C).

The “in vivo” treatment with gadolinium chloride effectively inhibited Kupffer cell phagocytic activity, as was shown by the net reduction of carbon clearance in the liver of both unpoisoned and haloalkane-treated animals (Fig. 4), and more clearly, by light microscopic analysis (Fig. 5D). Indeed, after treatment with the macrophage inhibitor, only endothelial cells were able to take up carbon particles (Fig. 5D).

After confirming the inhibition of Kupffer cell activity

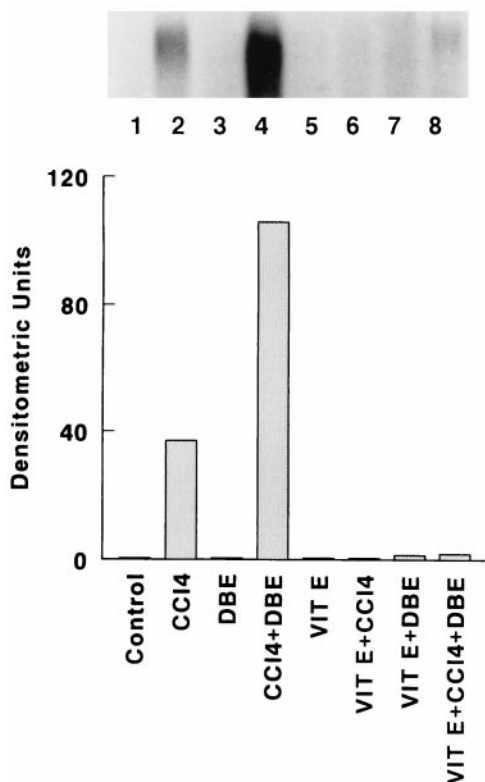


Fig. 3. Effect of vitamin E pretreatment on hepatic AP-1 binding activity, 6 h after poisoning with the haloalkane/s. Figure shows 1 of 2 consistent experiments. Top: autoradiogram of AP-1 binding to DNA in control (lanes 1–4) and vitamin E supplemented rats (lanes 5–8). See Methods for details. Lanes 1, 5: mineral oil; lanes 2, 6: 6 h after CCl₄ treatment; lanes 3, 7: 6 h after DBE treatment; lanes 4, 8: CCl₄ + DBE. Bottom: the bands shown in the top autoradiogram were quantitated by phosphorimaging and expressed in arbitrary densitometric units.

by GdCl₃, the effect of this drug on CCl₄-induced AP-1 upregulation was investigated. As reported in Fig. 6, the marked stimulation of hepatic AP-1-DNA binding following acute poisoning with CCl₄ or CCl₄ + DBE was consistently prevented when rats were pretreated with GdCl₃.

DISCUSSION

Induction of genes involved in tissue repair occurs soon after exposure to prooxidant necrogenic hepatotoxins. Acute treatment with carbon tetrachloride has been repeatedly shown to upregulate the expression of *c-fos* and *c-jun* genes, as well as the DNA binding of AP-1 transcription factor, in mice and rats [8,9,24–26]. The present report confirms the marked stimulation of AP-1 formation and binding after a single dose of CCl₄, but also shows that the effects of the drug on the hepatic redox state and on AP-1 binding are rather early events, well established when the biochemical footprint of irre-

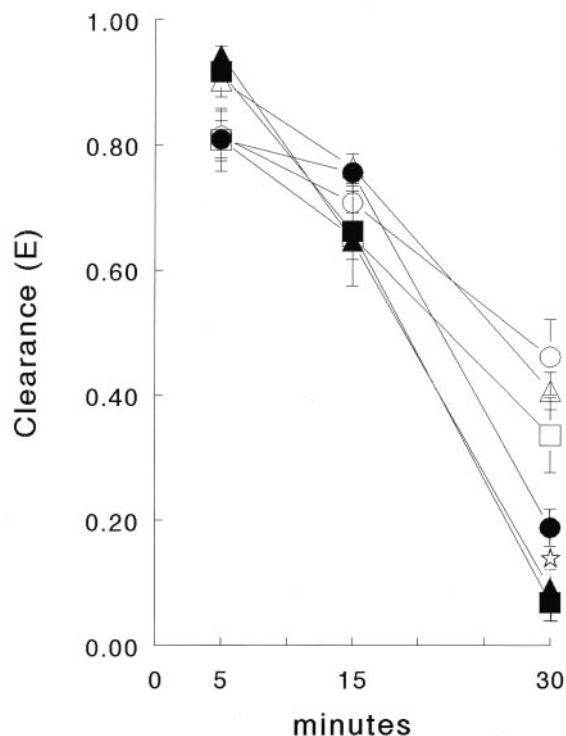


Fig. 4. Effect of gadolinium chloride on colloidal carbon clearance. Rats were injected with GdCl₃ or saline solution 24 h before being challenged with haloalkanes or vehicle only. After 6 h, they received colloidal carbon solution (100 mg/kg i.v.). Blood samples were collected at different times and hemolyzed. Changes in absorbance at 640 nm against a blank (blood collected before carbon injection) were used to calculate the removal rate of colloidal carbon from blood stream. ●: control; ▲: CCl₄; ■: CCl₄ + DBE; ○: GdCl₃; △: GdCl₃ + CCl₄; □: GdCl₃ + CCl₄ + DBE. Data are means ± SD of three to four different animals. ☆ Significant with respect to GdCl₃-treated rats.

versible cell damage is only just detectable. Moreover, the simultaneous treatment of the rats with a quantity of 1,2-dibromoethane that is certainly not necrogenic [7] produced a strong potentiating effect, both on the inducible extent of lipid peroxidation and on AP-1 upregulation, still with little evidence of cytolysis.

The parallel stimulation of lipid peroxidation and AP-1 binding by the synergistic mixture of haloalkanes, together with the inhibition of both effects by a large dose of vitamin E, point to a cause-effect relationship between the two events, even if mechanisms other than oxidant-dependent damage to the modulation of the transcription factor cannot be excluded.

To exert its toxic action, CCl₄ must be homolytically cleaved at the cytochrome P450 level, producing the trichloromethyl free radical (CCl₃·), which is then converted to the trichloromethylperoxy radical (CCl₃O₂·), which is believed to be the reactive species that triggers the oxidative breakdown of membrane polyunsaturated fatty acids [27,28]. The haloalkane metabolic cleavage is produced by the cytochrome P450 isoform 2E1 [29],

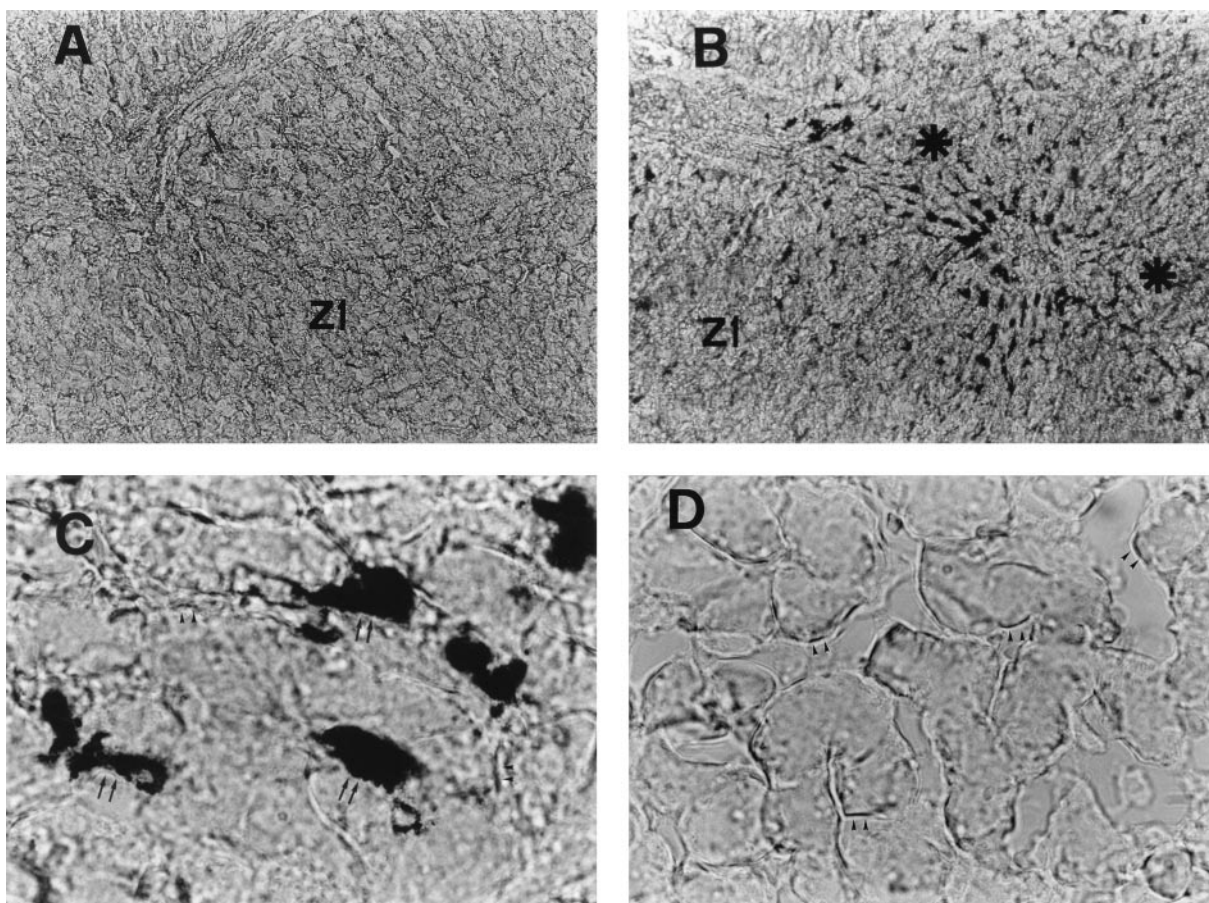


Fig. 5. Light microscopy analyses of Kupffer cell phagocytic activity after carbon administration. The study was performed on control and $\text{CCl}_4 + \text{DBE}$ treated rats, both preinjected and not preinjected with GdCl . Cryosections were from liver fragments taken from animals killed 60 min after colloid carbon administration (see Materials and Methods section). (A) Control rats not treated with GdCl ($\times 13$); (B) $\text{CCl}_4 \pm \text{DBE}$ -intoxicated rats not treated with GdCl ($\times 13$); (C) same experimental group at higher magnification ($\times 83$); (D) $\text{CCl}_4 \pm \text{DBE}$ intoxicated rats also pretreated with GdCl ($\times 83$). Z1: periportal zone of the liver acinus; single arrow: portal space; * periportal activated Kupffer cells; double arrows: activated Kupffer cells; double arrow-heads: sinusoidal endothelial cells containing carbon particles.

which is present in hepatocytes, especially in those of centrilobular area, and to a lower extent, in Kupffer cells [30].

Detoxification of DBE includes both direct conjugation with glutathione, catalyzed by glutathione transferase, and microsomal oxidation, again by the cytochrome P450 isoform 2E1 [31]. DBE metabolism by the latter pathway leads to the formation of bromoacetaldehyde, responsible for this haloalkane's recognized glutathione depleting action. Co-treatment with DBE, in addition to induction of cytochrome P450 after only a few hours' acute poisoning (Chiarpotto and Poli, unpublished results), increases the hepatocyte's susceptibility to CCl_4 -triggered membrane lipid oxidation [32]. In turn, CCl_4 exacerbates DBE's damaging action, by impairing its elimination via glutathione transferase, and thus, enhances the P450-dependent pathway and bromoacetaldehyde formation [32].

With regard to the molecular mechanisms of AP-1 upregulation by CCl_4 plus or minus DBE, many diffusible products stem from membrane lipid peroxidation, triggered by CCl_3O_2^* [33], or by DBE-derived reactive species [32] such as lipid peroxides, hydroperoxides, and aldehydes. The latter include saturated aldehydes, such as bromoacetaldehyde, propanal, and hexanal, but also, more importantly, certain hydroxyalkenals, with multiple effects that depend on their steady-state concentration [34,35]. Contrary to the more traditional view that their role is merely that of reactive species responsible for toxicity, these aldehydes at low concentrations are important in modulating several cellular functions [34,35]. A major hydroxyalkenal product of lipid peroxidation, 4-hydroxy-2,3-nonenal (HNE), has been shown to increase AP-1 nuclear levels in murine and human macrophages [5], as well as in human hepatic stellate cells (Parola, Camandola et al., submitted for publication). In

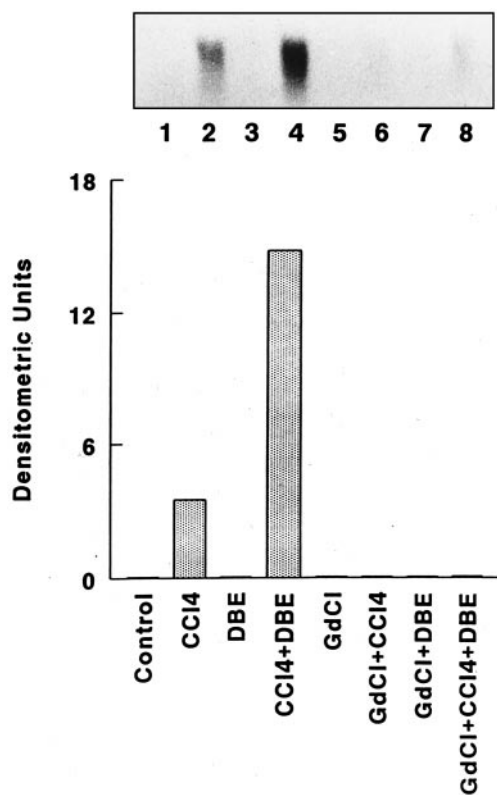


Fig. 6. Effect of gadolinium chloride pretreatment on rat liver AP-1 binding activity 6 h after poisoning with $\text{CCl}_4 \pm \text{DBE}$. Figure shows 1 of 2 consistent experiments. Top: AP-1-DNA binding in control (lanes 1–4) and gadolinium chloride-treated rats (lanes 5–8). Lanes 1, 5: mineral oil; lanes 2, 6: 6 h after CCl_4 treatment; lanes 3, 7: 6 h after DBE treatment; lanes 4, 8: $\text{CCl}_4 + \text{DBE}$. Bottom: the bands shown in the top autoradiogram were quantitated by phosphorimaging and expressed in arbitrary densitometric units.

addition, HNE stimulates expression and synthesis of the AP-1 dependent genes collagen type I [36] and transforming growth factor $\beta 1$ [37].

The fact that $\text{CCl}_4 \pm \text{DBE}$ -induced activation of AP-1 can be suppressed by giving the animals a dose of α -tocopherol, shown to quench CCl_4 -stimulated hydroxyalkenal production [10], means that a causative role of diffusible lipid peroxidation products (aldehydes, etc.) in the genesis of the AP-1 increase is a viable possibility.

With regard to the cell types presumably involved, together with hepatocytes, in bringing about the observed marked AP-1 nuclear binding, Kupffer cells are primary candidates. GdCl blocks phagocytosis by rat Kupffer cells, and selectively eliminates the large Kupffer cells in zone I, the periportal region of the acinus [38]. Rat pretreatment with this inhibitor practically abolishes the AP-1 response to haloalkanes found at 6-h poisoning, when morphological analyses still excluded any inflammatory infiltrate in the area (Figs. 5C, 5D).

It may be suggested that activation of hepatic macro-

phages by in loco lipid peroxidation products is the prerequisite for activation of Kupffer cells and subsequent recruitment of all other possible cells.

In a previous study, CCl_4 -dependent activation was found for phospholipase A2 [39,40], the enzyme providing free arachidonate for both oxidative breakdown and metabolism. Very recently, the stimulation of eicosanoid synthesis by CCl_4 was carefully analyzed by Johnston and Kroening in cultured rat liver cells [41]; they showed that arachidonic acid was detectable in the culture medium of poisoned cells only when hepatocytes were cultured with non-parenchymal cells. Whether the latter are the source of arachidonic acid, or are necessary for the hepatocytes to release arachidonate, is not yet clear.

While the interaction between hepatocytes and Kupffer cells is still unclear in biochemical terms, arachidonic acid may well be the chief source of active chemical mediators in the very early phases of poisoning, when it undergoes oxidative breakdown, as is clearly demonstrated by the increased liver malonaldehyde (TBARS) production.

In summary, in rats poisoned with a very low dose of CCl_4 , we have obtained evidence of a marked enhancement of AP-1 binding activity in the liver prior to the appearance of overt cytotoxic effects. Another important conclusion is that AP-1 upregulation is dependent upon the stimulating effect of the haloalkane on membrane lipid peroxidation. By using an antioxidant dose of α -tocopherol, or increasing lipid peroxidation with the DBE- CCl_4 synergistic mixture, the effect of CCl_4 on AP-1 binding can be prevented or further stimulated, respectively.

With regard to the mechanisms involved in AP-1 modulation, the early increase in membrane lipid peroxidation consequent on metabolic activation of CCl_4 represents a readily available source of diffusible intermediates for further cell recruitment. Kupffer cell activity also appears essential for AP-1 upregulation, probably due to its role in the release of arachidonate during intoxication. Among the several secretory products of hepatic macrophages, some are probably involved in the long-term modulation of genes related to the remodeling and proliferation that follow tissue damage. But aldehydes, and possibly other products of arachidonic acid oxidative breakdown, appear to be the molecules most involved in bringing about the AP-1 response in the early phases of acute CCl_4 poisoning.

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ABBREVIATIONS

CCl₄—carbon tetrachloride
DBE—1,2-dibromoethane
AP-1—activator protein 1
SDH—sorbitol dehydrogenase
GPT—glutamic-pyruvic-transaminase
GOT—glutamic-oxalacetic-transaminase
GdCl—gadolinium chloride
TBARS—thiobarbituric acid reacting substances
HNE—4-hydroxy-2,3-nonenal
ROS—reactive oxygen species