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# Oxidative stress-mediated hepatotoxicity of iron and copper: Role of Kupffer cells

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**Abstract**

Iron- or copper-mediated catalysis leads to the generation of reactive oxygen species (ROS) that can attack biomolecules directly, with the consequent enhancement in membrane lipid peroxidation, DNA damage, and protein oxidation. Reactive nitrogen species (RNS) can also be formed, leading to nitration of aromatic structures in addition to the oxidative deterioration of cellular components. Kupffer cells, the resident macrophages of the liver, play significant roles in immunomodulation, phagocytosis, and biochemical attack. Upon stimulation, liver macrophages release biologically active products related to cell injury, namely, ROS, RNS, and both immunomodulatory and fibrogenic cytokines, with production of chemokines and adhesion molecules by other cells of the liver sinusoid. Iron and copper alter the functional status of Kupffer cells by enhancing their respiratory burst activity without modifying particle phagocytosis. This effect is probably due to extra $O_2$ equivalents used in the oxidation of biomolecules and/or in the activating action of iron/copper on nitric oxide synthase, in addition to those employed by NADPH oxidase activity. Changes in gene expression of Kupffer cells may also be accomplished by iron and copper through modulation of the activity of transcription factors such as NF-$\kappa$B, which signals the production of cytotoxic, proinflammatory, or fibrogenic mediators. Thus, iron/copper-induced hepatotoxicity is a multifactorial phenomenon underlying actions due to the generation of ROS and RNS that may alter essential biomolecules with loss of their biological functions, modulate gene expression of Kupffer cells with production of cytotoxic mediators, or both.

## Oxidative mechanisms in the toxicity of iron and copper

Transition metals such as iron and copper are implicated in a number of physiological, toxicological, and pathological processes due to their capacity to undergo changes of oxidation states involving electron transfer (Aust *et al.* 1985; Okada 1998). In aerobic cells, electron transport from metabolic fuels to $O_2$ is carried out through a series of iron and iron/copper containing respiratory complexes located in the inner mitochondrial membrane. The free energy released is used in part to pump $H^+$ across the inner membrane, thus establishing an electrochemical proton gradient that allows ATP production by the adenosine-5′-triphosphate synthase complex (Videla 2000).

Iron- and copper-catalysis can also cause formation of reactive $O_2$ species in cells, provided that a suitable concentration of the free redox-active transition metals is available (Aust *et al.* 1985; Luza & Speisky 1996). In fact, both ferric ($Fe^{3+}$) and cupric ($Cu^{2+}$) ions at physiological concentrations can promote the generation of hydroxyl radical ($HO^{\bullet}$), or a species of equivalent reactivity, in a reaction requiring a reducing agent (i.e., superoxide radical [$O_2^{\bullet -}$]) and hydrogen peroxide ($H_2O_2$) known as the iron/copper-catalyzed Haber-Weiss reaction (Haber & Weiss 1934; Samuni *et al.* 1981). The latter reaction involves the oxidation of $Fe^{2+}$ or $Cu^{+}$ by $H_2O_2$ that gen-

erates $HO^{\bullet}$ and/or ferryl species (Fenton's reaction) (Fenton 1894; Kozlov & Berdnikov 1973) which, in turn, may initiate free-radical chain reactions with several target biomolecules when produced in close proximity to them. Thus, iron/copper-induced reactive $O_2$ species generation may lead to (i) formation of polyunsaturated fatty acid-derived hydroperoxides in membrane phospholipids, that can undergo decomposition in the presence of iron/copper ions into additional free-radical moieties (lipid peroxidation) (Aust *et al.* 1985); (ii) DNA damage by point mutations, DNA cross-linking, and/or DNA strand breaks (Halliwell & Aruoma 1993); (iii) oxidative modifications in side chains of amino acid residues in protein (protein oxidation) (Stadtman 1990); and (iv) depletion of sulfhydryls and alteration of calcium homeostasis (Stohs & Bagchi 1995). In addition to formation of reactive $O_2$ species, it has been shown that the interaction of chelates of iron and copper with peroxynitrite ($ONOO^-$) promotes its heterolytic cleavage to yield a species with the reactivity of nitronium ion ($NO_2^+$), a strong oxidizing species that can also lead to the nitration of aromatic structures such as tyrosine (Beckman *et al.* 1992). However, nitric oxide ($^{\bullet}NO$) can modulate free radical processes through (i) binding of $Fe^{2+}$ with production of an iron-nitrosyl complex and diminution of iron availability for Fenton-mediated processes (Radi *et al.* 1995); or (ii) binding of $Fe^{3+}$ or higher oxidation states of iron, with the consequent inhibition of related oxidative reactions (Radi *et al.* 1995). Thus, the net cytotoxic potential of iron/copper-induced oxidative mechanisms will depend on critical factors such as the type and redox capacity of the transition metal species involved, as well as the relative concentrations of reactive oxygen and nitrogen species at specific cell compartments.

The liver plays a central role in the maintenance of body iron and copper homeostasis; however, excess deposition of the transition metals occurs upon overload, leading to hepatocellular injury and functional insufficiency (Bacon & Britton 1989; Linder & Hazegh-Azam 1996). The propensity of iron/copper for catalyzing production of reactive oxygen and nitrogen species represents a major molecular mechanism triggering hepatocellular-damaging effects, which become significant when the capacity of the liver to maintain these metals in storage forms is exceeded.

**The role of Kupffer cells in liver injury**

Kupffer cells are resident macrophages of the liver playing significant roles in immunomodulation, phagocytosis, and biochemical attack (Decker 1990). Uptake of particles by Kupffer cells is mediated by plasma membrane receptors, with the concomitant release of specific molecules including proteases, bioactive lipids, cytokines, and reactive oxygen and nitrogen species (Decker 1990; Wang *et al.* 1993). Among the latter species, $O_2^{\bullet -}$ is predominantly formed in the respiratory burst of activated Kupffer cells (Wang *et al.* 1993), a phenomenon that involves the protein kinase C-dependent activation of NADPH oxidase (Decker 1990). Liver macrophages also produce $^{\bullet}NO$ by the inducible isoform of NO synthase (NOS2), at rates of about one eighth of those of $O_2^{\bullet -}$ (Wang *et al.* 1993). In this situation, formation of $ONOO^-$ may occur by the $^{\bullet}NO$-$O_2^{\bullet -}$ combination reaction known to proceed at an almost diffusion-controlled rate (Padmaja & Huie 1993), as shown in activated alveolar lung macrophages (Ischiropoulos *et al.* 1992). In addition to reactive species, activated Kupffer cells are known to produce and release various mediators related to liver injury, namely, cytokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interferon $\alpha/\beta$, and interleukin (IL) -1 and -6 (Decker 1990). TNF-$\alpha$ is considered to be a common early effector molecule for liver injury, considering that, in addition to its direct cytotoxic effects, this cytokine is able to induce chemokines (IL-8, macrophage inflammatory protein-1$\alpha$ [MIP-1], macrophage chemotactic protein-1 [MCP-1]) and adhesive molecules (intercellular adhesion molecule-1 [ICAM-1], vascular-cell adhesion molecule-1 [VCAM-1]), which are key to inflammation and consequent liver damage (Tsukamoto & Lin 1997).

Experimental evidence supports the pathogenic role of Kupffer cells in liver injury induced by endotoxin, xenobiotics such as acetaminophen, ethanol, and carbon tetrachloride, ischemia-reperfusion (Tsukamoto & Lin 1997), or hyperthyroid state (Tapia *et al.* 1997). In fact, prevention of liver injury has been observed upon (i) elimination of Kupffer cells by gadolinium chloride ($GdCl_3$); (ii) neutralization of TNF-$\alpha$ with anti-TNF-$\alpha$ antibody; (iii) prevention of translation of primary RNA transcript of TNF-$\alpha$ by antisense oligonucleotide; and (iv) interaction of TNF-$\alpha$ with soluble TNF-$\alpha$ receptors (Van Zee *et al.* 1992; Tsukamoto & Lin 1997; Tu *et al.* 1998).

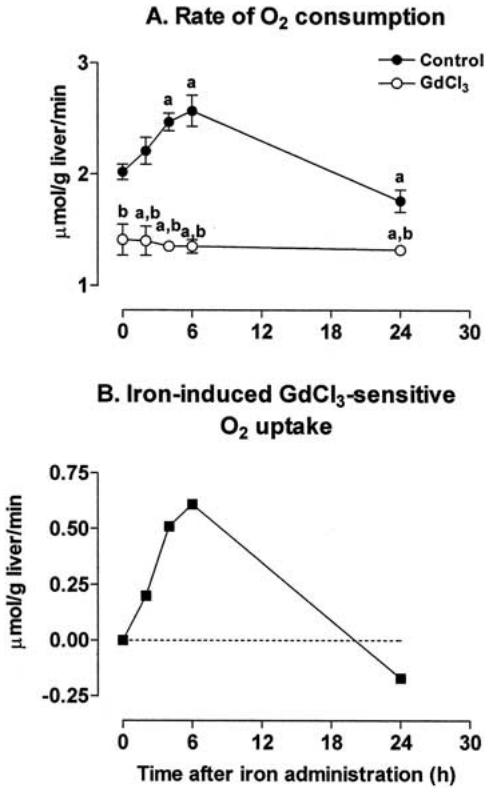


*Fig. 1.* Time course study of the effect of $GdCl_3$ pretreatment on the rate of $O_2$ consumption in isolated perfused livers from control rats and iron-overloaded animals (A) and iron-induced $GdCl_3$-sensitive $O_2$ uptake (B). Male Sprague-Dawley rats weighing 150-180 g were given free access to food and water and maintained in a 12 h light/dark cycle. Animals were given either $GdCl_3$ (10 mg kg iv) or equivalent volumes of 0.9% wt vol NaCl 24 h before acute iron overload (500 mg kg ip), and studies were carried out at 0 (controls), 2, 4, 6, and 24 h after treatment. Livers were perfused via the portal vein in a non-recirculating system as described previously (Tapia *et al.* 1997), using Krebs-Henseleit bicarbonate buffer saturated with 95% $O_2$/5% $CO_2$ to give pH 7.4, at constant flow rates (3.5–4.0 ml g liver min) and temperature (36–37 °C). To assess the net effect of $GdCl_3$ on liver $O_2$ consumption shown in (B), rates of $O_2$ uptake in $GdCl_3$-pretreated rats were subtracted from those in animals not treated with the Kupffer cell inactivator and were corrected for the decrease observed in rats not treated with iron. This latter effect of $GdCl_3$ is possibly caused by depression of mitochondrial respiration (Ferreira *et al.*1998). Results represent means ± SEM for four to seven animals per group. The statistical significance of differences between mean values was assessed by one-way ANOVA and the Newman-Keuls, test: (a) $P < 0.05$ compared with control rats (time zero); (b) $P < 0.05$ compared with the respective group of control and iron-overloaded animals without $GdCl_3$ pretreatment. Modified from Tapia *et al.* (1998).

**Kupffer cell functioning and hepatotoxicity after acute iron overload *in vivo***

Acute iron overload in rats leads to an alteration in Kupffer cell functioning, shown by an early enhancement in the rate of $O_2$ consumption of the liver that is suppressed by $GdCl_3$ (Figure 1A). In fact, liver $GdCl_3$-sensitive respiration is progressively increased by iron overload in the absence of an additional stimulus, reaching a maximum at 6 h after treatment and then decreasing to below control values at 24 h (Figure 1B). This effect of iron accounts for all the net increase in total hepatic $O_2$ uptake and is probably associated with $O_2$ equivalents used in the respiratory burst activity of Kupffer cells that involves generation of reactive oxygen and nitrogen species (Decker 1990).

Kupffer cell functioning can be monitored continuously by the infusion of colloidal carbon into the isolated perfused rat liver (Figure 2A), which leads to a significant uptake of the particles exclusively by non-parenchymal cells and predominantly by Kupffer cells (Cowper *et al.* 1990). Carbon phagocytosis is paralleled by an enhancement in the rate of $O_2$ consumption of the liver over basal values (Figure 2B), which is largely accounted for by the respiratory burst of Kupffer cells (Decker 1990; Wang *et al.* 1993). Minor mitochondrial respiratory components include $O_2$ uptake in Kupffer cells for energy supply needed for carbon phagocytosis (Cowper *et al.* 1990) or in hepatocytes, possibly mediated by prostaglandins released by activated liver macrophages (Qu *et al.* 1996). Integration of the area under the carbon uptake and $O_2$ consumption curves gives the total carbon uptake (23.5 mg g liver; Figure 2A) or total carbon-induced $O_2$ consumption (2.32 $\mu$mol g liver; Figure 2B) in the 15 min time interval studied, respectively, integrated values that allow the calculation of the respective $O_2$/carbon uptake ratios (0.099 $\mu$mol $O_2$ mg carbon) (Tapia *et al.* 1997).

Carbon uptake by the perfused liver was not modified by iron overload, whereas the selective inactivation of Kupffer cells by $GdCl_3$ led to 62% to 76% diminution in carbon phagocytosis, both in controls and in iron-overloaded animals (Figure 3A). This may be due to the finding that $GdCl_3$ eliminates only large Kupffer cells and depresses carbon phagocytosis of small liver macrophages, whereas that of endothelial cells is increased (Hardonk *et al.* 1992). Iron overload elicited a time-dependent biphasic effect on $GdCl_3$-sensitive carbon-induced $O_2$ uptake (Figure 3B) that

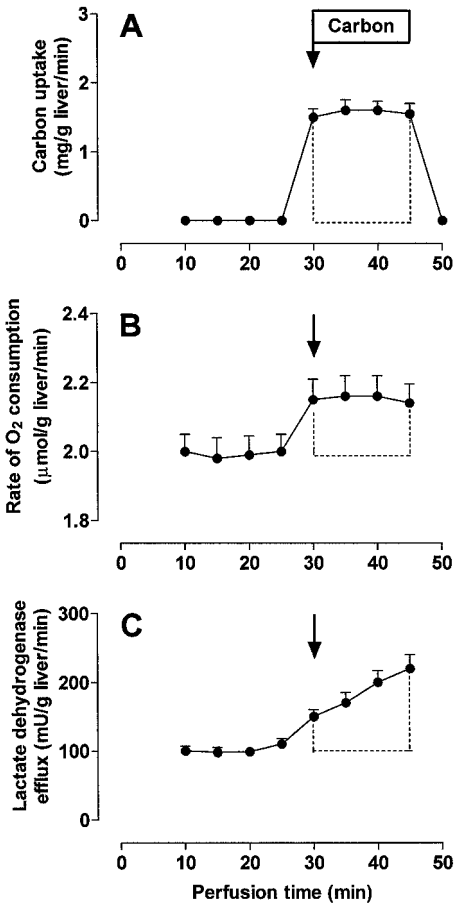


*Fig. 2.* Experimental design to assess the influence of colloidal carbon infusion (0.5 mg ml) on carbon uptake (A), $O_2$ consumption (B), and sinusoidal LDH efflux (C) by isolated perfused rat livers. Male Sprague-Dawley rats weighing 150–180 g were given free access to food and water and maintained in a 12-h light/dark cycle. Livers were perfused as described in Figure 1. (A) In order to determine carbon uptake rates, the absorbance at 623 nm ($A_{623}$) was measured at 5-min intervals during carbon infusion (30- to 45-min time interval)(Cowper *et al.* 1990). Rates of carbon uptake (in mg g liver min) were calculated from the influent minus effluent differences in $A_{623}$, the specific extinction coefficient for carbon at 623 nm (0.97[mg ml]$^{-1}$), and the perfusion flow. Total carbon uptake was obtained by the integration of the respective curves between 30- to 45-min perfusion and expressed as mg g liver [$23.5 \pm 2.0$ ($n = 5$) mg g liver]. (B) Carbon-induced $O_2$ uptake was estimated by the integration of the area under the $O_2$ consumption curves from 30- to 45-min perfusion with carbon and expressed as μmol/g liver [$2.32 \pm 0.1$ ($n = 5$) μmol g liver]. Total LDH efflux in the presence of carbon was calculated by the integration of the area under the net sinusoidal release (mU g liver min) curve from 30- to 45-min perfusion and expressed as U/g liver [$0.099 \pm 0.01$ ($n = 5$) U/g liver]. Results represent the means ± SEM for five animals.

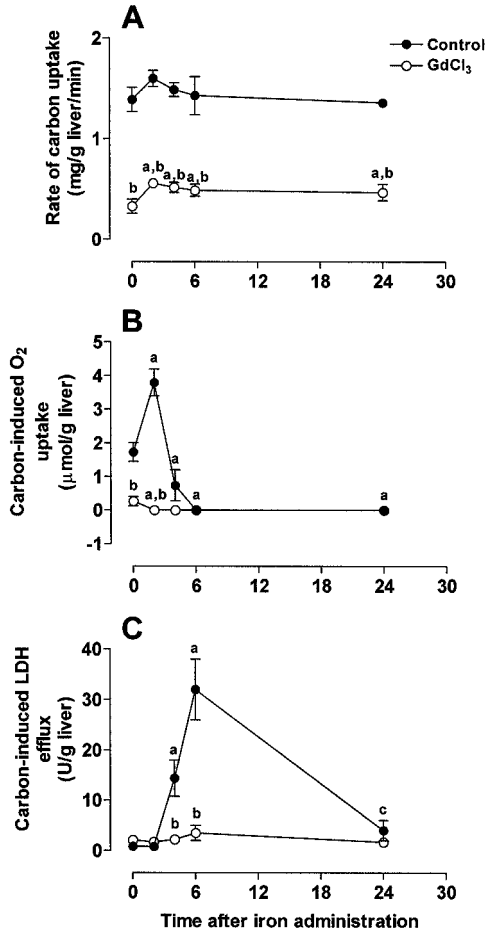


*Fig. 3.* Time course of the effect of $GdCl_3$ pretreatment on the rate of carbon uptake (A), carbon-induced $O_2$ uptake (B), and sinusoidal carbon-induced LDH efflux (C) by perfused livers from control and iron-overloaded rats. Male Sprague-Dawley rats were given either $GdCl_3$ (10 mg kg iv) or equivalent volumes of 0.9% wt/vol NaCl 24 h before acute iron overload (500 mg kg ip), and studies were carried out at 0 (controls), 2, 4, 6, and 24 h after treatment. Livers were perfused as described in Figures 1 and 2. Results represent means ± SEM for four to seven animals per group. The statistical significance of differences between mean values was assessed by one-way ANOVA and the Newman-Keuls, test: (a) $P < 0.05$ compared with control rats (time zero); (b) $P < 0.05$ compared with the respective group of control and iron-overloaded animals without $GdCl_3$ pretreatment; (c) $P < 0.05$ compared with the carbon-induced LDH efflux at 6 h after iron overload. Modified from Tapia *et al.* (1998).

seems to depend on the magnitude of the enhancement in Kupffer cell respiratory activity elicited by the *in vivo* iron treatment (Figure 1B). At 2 h after iron overload, carbon-induced Kupffer cell-dependent respiration is increased by 119% (Figure 3B) in conditions in which the iron-induced $GdCl_3$-sensitive $O_2$ uptake is elevated by only 9% (Figure 1B). When the

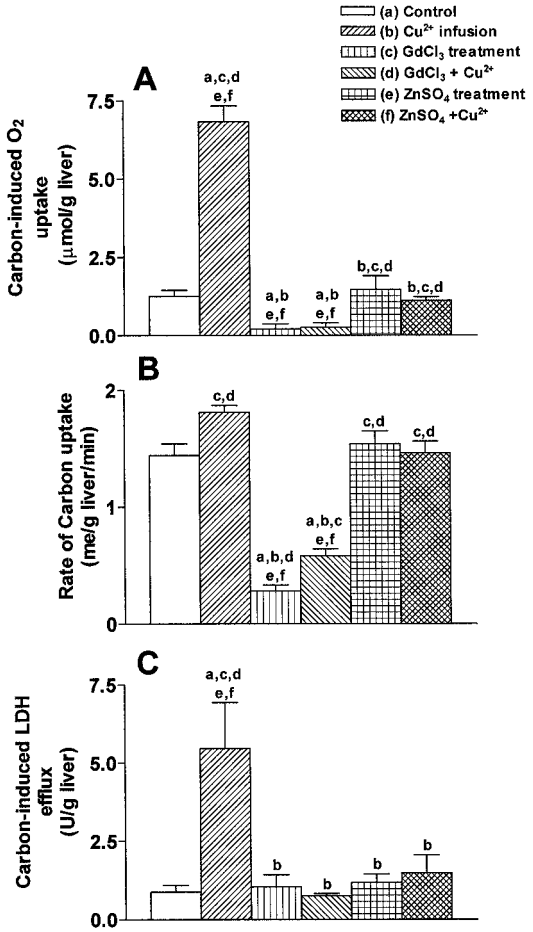


*Fig. 4.* Concentration-dependent effects of $Cu^{2+}$ on the rate of carbon uptake and carbon-induced $O_2$ uptake (A), and the respective $O_2$/carbon uptake ratios (B) by isolated perfused rat liver. Livers were perfused as described in Figure 1, in the absence ($Cu^{2+}$ concentration = 0) or presence of 0.1, 0.25, 0.5, and 1.0 $\mu$M $Cu^{2+}$ infused at 20 min perfusion and subjected to 0.5 mg carbon ml according to Figure 2. Oxygen/carbon uptake ratios were calculated by dividing the respective integrated values obtained between 30–45 min perfusion. Results represent means ± SEM for four to six animals per group. The statistical significance of differences between mean values was assessed by one-way ANOVA and the Newman-Keuls, test: $^{a}P < 0.05$ compared with controls (zero $Cu^{2+}$ concentration); $^{b}P < 0.05$ compared with 0.1 $\mu$M $Cu^{2+}$. Modified from Sans *et al.* (1999).

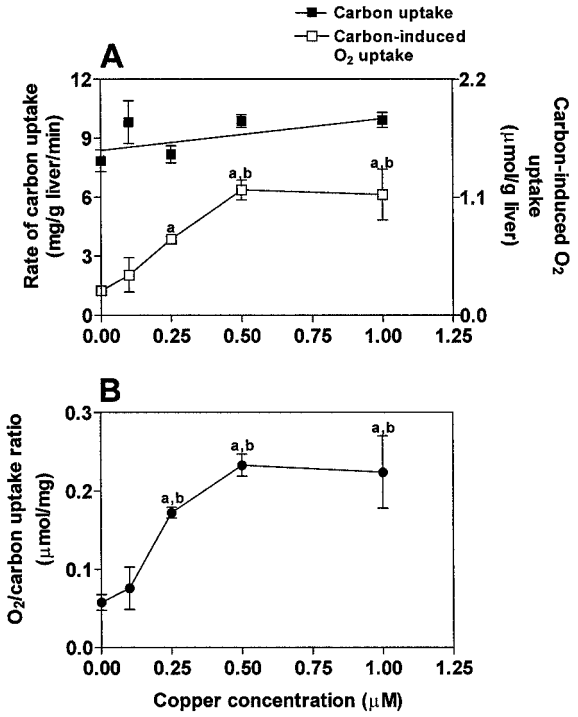


*Fig. 5.* Effect of gadolinium chloride ($GdCl_3$) and zinc sulfate ($ZnSO_4$) pretreatments on the rate of carbon uptake (A), carbon-induced $O_2$ uptake (B), and carbon-induced LDH efflux (C) by rat livers perfused in the absence (controls) and presence of 0.5 $\mu$M $Cu^{2+}$. Studies were carried out in separate groups of rats, 24 h after pretreatment with $GdCl_3$ (10 mg kg iv), 17 h after pretreatment with $ZnSO_4$ (three doses of 10 mg kg ip equally spaced in a 24-h time interval), or equivalent volumes of 0.9% wt/vol NaCl (controls). Liver perfusion and Kupffer cell functioning were performed as described in Figures 1 and 2, respectively. Results represent means ± SEM for four to six animals per group. The statistical significance of differences between mean values ($P < 0.05$) was assessed by one-way ANOVA and the Newman-Keuls' test, and is shown by the letters identifying each experimental group. In separate groups of control rats and $ZnSO_4$-treated animals, the content of hepatic GSH was determined by the catalytic assay of Tietze (1969) [controls, 5.59 ± 1.38 $\mu$mol g liver ($n = 3$); $ZnSO_4$ treatment, 7.02 ± 0.35 ($n = 3$); not significant] and that of metallothionein was assessed according to Eaton & Toal (1982) [controls, 1.2 ± 0.4 nmol g liver ($n = 3$), $ZnSO_4$ treatment, 108.2 ± 39.2 ($n = 3$); $P < 0.025$]. Modified from Sans *et al.* (1999).

latter respiratory component induced by iron is enhanced to 21% and 24% of the total $O_2$ consumption at 4 and 6 h after treatment (Figure 1B), the respective $GdCl_3$-sensitive carbon-induced respiration is either decreased compared to control values at 4 h or abolished at 6 h (Figure 3B). Iron overloaded rats exhibit liver injury as shown by the substantial increase in the carbon-induced sinusoidal release of lactate dehydrogenase (LDH) by perfused livers observed at 4 (15-fold), 6 (31-fold), and 24 (3.5-fold) h after treatment (Figure 3C). This effect of iron coincides with the Kupffer cell-dependent respiratory activity induced (Figure 1B) and is characterized by being markedly diminished by Kupffer cell depletion (Figure 3C). Thus, iron-induced hepatotoxicity occurs at early times after treatment and is largely dependent on the activity of Kupffer cells through promotion of free-radical reactions associated with the respiratory

burst. Furthermore, carbon-induced liver LDH release was diminished significantly at 24 h after iron overload compared with values found at 6 h (Figure 3C). This effect is observed concomitantly with a small (13%) but significant decrease in the basal rate of $O_2$ consumption of the liver compared with control values (Figure 1A), without development of the iron-induced $GdCl_3$-sensitive respiratory component (Figure 1B) or liver macrophage respiratory activation by carbon infusion (Figure 3B). Collectively, these data indicate that iron overload at 24 h after administration leads to an impairment of Kupffer cell functioning, probably related to the excessive pro-oxidant activity induced at earlier times, that may lead to inactivation of NADPH oxidase (Jandl *et al.* 1978), damage to liver macrophages and other cell types (Kaplan *et al.* 1975), and derangement of respiratory processes in hepatocytes (Figure 1A). Iron-induced pro-oxidant activity has been proposed as a major factor in the impairment of the chemotactic, phagocytic, and bactericidal capacity of neutrophils from patients with iron overload who exhibit an increased risk of developing bacterial infections (Weinberg 1978; Van Asbeck *et al.* 1984), or in the detrimental effects of the transition metal that accumulates in sinusoidal lining cells and Kupffer cells in hepatitis virus infection (Bonkovsky *et al.* 1997).

### Kupffer cell functioning and hepatotoxicity after copper overload *in vitro*

Colloidal carbon stimulation of Kupffer cells in the perfused rat liver in the presence of $Cu^{2+}$ elicited a concentration-dependent sigmoidal enhancement in $O_2$ consumption, with a half-maximal concentration of 0.23 $\mu$M (Figure 4A). A similar kinetic pattern is observed for the carbon-induced $O_2$ uptake/carbon uptake ratios at different $Cu^{2+}$ concentrations (Figure 4B), which, in addition to the lack of changes of $Cu^{2+}$ on particle phagocytosis (Figure 4A), suggest that $Cu^{2+}$ promotes $O_2$-dependent processes associated with the respiratory burst of activated Kupffer cells. Primarily, this phenomenon may involve reactive $O_2$ species generated by liver macrophage NADPH oxidase activity stimulated by carbon infusion, however, enhanced reactive nitrogen species formation cannot be discarded as $Cu^{2+}$ is known to effectively activate NOS (Ohnishi *et al.*1997; Plane *et al.*1997). The above contention is strongly supported by the abolishment of the $Cu^{2+}$-induced respiratory activity of activated liver macrophages by either Kupffer cell elimination by $GdCl_3$ or metallothionein induction by $ZnSO_4$ pretreatment (Figure 5A). Enhancement of hepatic metallothionein levels (Figure 5) is known to occur both in parenchymal cells as well as in endothelial and Kupffer cells (McKim *et al.* 1992), and represents a maximal copper binding capacity of 1284 nmol $Cu^{2+}$/g liver ([107 nmol metallothionein/g liver] $\times$ [12 nmol $Cu^{2+}$/nmol metallothionein]) (from Figure 5 and Sato & Bremmer 1993, respectively), that largely accounts for all the $Cu^{2+}$ infused into the liver (50 nmol $Cu^{2+}$/g liver, calculated considering a concentration of 0.5 $\mu$M $Cu^{2+}$ infused at 4 ml g liver min for 25 min). In addition, metallothionein is an effective free radical scavenger due to the high rate constants for reaction between its cysteinyl residues and oxyradicals (Sato & Bremmer 1993). In fact, metallothionein-induction by $ZnSO_4$ leads to a net 63% increase in the content of hepatic sulfhydryl groups over control values (control rats, $5618 \pm 1393$ nmol g liver ($n = 3$); $ZnSO_4$-pretreated rats, $9184 \pm 533$ ($n = 3$); $P < 0.05$), calculated by the sum of the nmol of glutathione (GSH)/g liver and nmol of [(metallothionein/g liver) $\times$ 20] (from Figure 5) considering that one molecule of metallothionein contains 20 cysteinyl residues (Sato & Bremmer 1993). This effect may contribute to the normalization of the $Cu^{2+}$-induced enhancement in the carbon-dependent respiratory burst of Kupffer cells (Figure 5A), in conditions that the rate of particle-phagocytosis is not altered (Figure 5B). $Cu^{2+}$-induced exacerbation of the respiratory activity of carbon-stimulated Kupffer cells is paralleled by a 5.2-fold increase in the sinusoidal efflux of LDH compared to control values (Figure 5C). This effect of $Cu^{2+}$ seems to be dependent on Kupffer cell functioning, due to its $GdCl_3$-sensitivity, and on the availability of the metal ion at the macrophage level, due to its abolishment by metallothionein induction (Figure 5C). Metallothionein induction by zinc is known to protect the liver against $Cu^{2+}$-induced hepatotoxicity by storing $Cu^{2+}$ in a nontoxic form (Lee *et al.* 1989; Schilsky *et al.* 1989), thus reducing the $Cu^{2+}$-dependent promotion of free radical processes leading to lipid peroxidation (Filipe *et al.* 1995), as reported for $\alpha$-tocopherol (Sokol *et al.* 1996). In line with the data presented, the pro-oxidant activity of neutrophils from patients in the active stage of Behcet's disease has been ascribed to an enhanced activity of the $O_2^{\bullet -}$ generator NADPH oxidase that may be exacerbated by the elevated levels of plasma copper found (Dogan *et al.* 1994), leading to a depression of the antioxidant defenses of plasma with

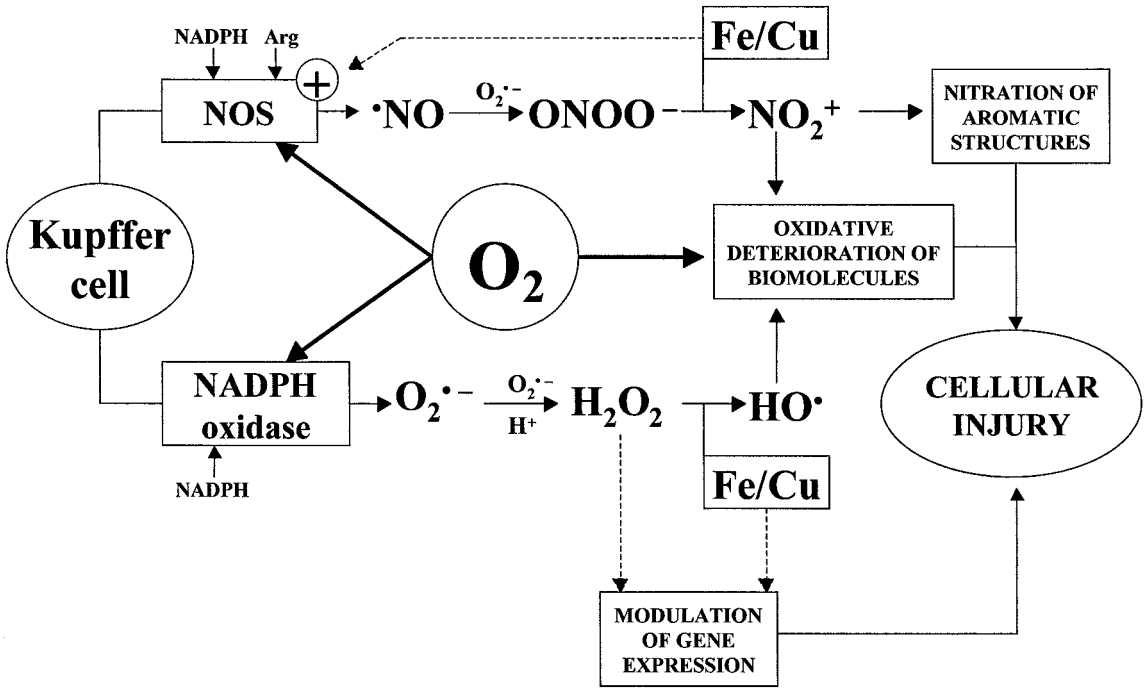


*Fig. 6.* Involvement of iron/copper in the respiratory burst activity of Kupffer cells and its functional and cytotoxic consequences. Abbreviations: Arg – L-arginine; NOS – nitric oxide synthase; $^{\bullet}NO$ – nitric oxide; $O_2^{\bullet-}$ – superoxide radical; $ONOO^-$ – peroxynitrite ion; $H_2O_2$ – hydrogen peroxide; $NO_2^+$ – nitronium ion; $HO^{\bullet}$ – hydroxyl radical.

the consequent increase in lipid peroxidation indexes (Kose *et al.* 1995).

## Concluding remarks

Iron and copper alter the functional status of Kupffer cells by inducing secondary reactions related to the respiratory burst of activated liver macrophages (Figure 6), without a significant modification of particle phagocytosis. The effect of iron overload occurs at earlier times after administration, is sensitive to macrophage inactivation by $GdCl_3$, and seems to produce the impairment in the respiratory response of Kupffer cells to particle stimulation and in the basal hepatic respiration at later times after treatment. Low levels of copper also increase the Kupffer cell-dependent $O_2$ uptake, an effect that is abolished by macrophage elimination and metallothionein induction. Transition metal-induced Kupffer cell-dependent respiratory activity plays a role in the development of liver injury, assessed by the increased sinusoidal release of LDH, probably by catalyzing the generation of the secondary reactive species $HO^{\bullet}$ and $NO_2^+$ (Figure 6). These species are able to provoke oxidative modifications in biomolecules, thus consuming extra $O_2$ equivalents that contribute to the enhanced respiratory burst activity observed, and/or the nitration of aromatic structures, with the consequent alteration or loss of their biological functions. Kupffer cell-dependent respiration may also involve additional $O_2$ equivalents used in the reaction catalyzed by NOS (Figure 6), as NOS seems to be directly activated by iron (Cornejo *et al.* 2001) or copper (Ohnishi *et al.* 1997; Plane *et al.* 1997). Furthermore, modulation of gene expression in Kupffer cells either by iron or iron-induced oxidative stress may be involved in the hepatotoxic effects of the transition metal (Figure 6). Oxidative stress is considered as a major mechanism leading to the activation of transcription factors such as NF-$\kappa$B (Schreck *et al.* 1991), that signal an increased synthesis and release of cytotoxic, proinflammatory, or fibrogenic mediators (Decker 1990; Tsukamoto & Lin 1997). The pivotal role of iron in NF-$\kappa$B activation and expression of proinflammatory genes in Kupffer cells was demonstrated by iron chelation both in cholestatic (Lin *et al.* 1997) and alcoholic (Tsukamoto *et al.* 1999) liver injury. Similar observations were reported in human immunodeficiency virus-1 infection (Sappey *et al.* 1995), lung inflammation by particulate

air pollution (Jimenez *et al.* 2000), and in asbestos-induced fibrosis (Dai & Churg 2001). Although iron increases TNF-$\alpha$ release from human mononuclear cells (Muñoz *et al.* 1999), and iron deficiency reduces production of TNF-$\alpha$ in the same model (Muñoz *et al.* 1999) or that of interleukin 1 in rat leukocytes (Helyar & Sherman 1987), the mechanisms involved in these effects of iron status remain to be elucidated. Contrary to these effects of iron, copper suppresses TNF expression in murine macrophages by blocking NF-$\kappa$B activation, which in turn is produced by inhibition of I$\kappa$B kinase, acting as a thiol-reactive metal ion (Jeon *et al.* 2000). Down-regulation of NF-$\kappa$B by copper has been confirmed in different experimental models (Iseki *et al.* 2000; Zhai *et al.* 2000). Thus, iron/copper-induced hepatotoxicity is a multifactorial phenomenon underlying actions exerted through secondary reactive oxygen and nitrogen species. These mediators achieve cytotoxicity by either a direct alteration of essential biomolecules and/or an indirect modulation of liver macrophage gene expression (Figure 6).

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