Effects of the Kupffer cell inactivator gadolinium chloride on rat liver oxygen uptake and content of mitochondrial cytochromes

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Abstract The effect of gadolinium chloride ($GdCl_3$) on the content of rat liver mitochondrial cytochromes was investigated in relation to the basal rate of O_2 uptake and Kupffer cell functioning, assessed in liver perfusion studies. (1) A single dose of $GdCl_3$ (10 mg/kg) produced a significant diminution in Kupffer cell functioning, evidenced by the decreases in colloidal carbon uptake and in carbon-induced O_2 uptake observed at 6–24 h after treatment, without changes in the sinusoidal lactate dehydrogenase efflux as index of tissue viability; at 48 h after $GdCl_3$ administration, carbon phagocytosis was recovered to control values, whereas carbon-induced O_2 uptake remained lower than control values. (2) $GdCl_3$ also caused a 34% decrease in the basal rate of O_2 consumption of the liver at 24 h after treatment, which returned towards control values at 48 h. (3) The content of mitochondrial cytochromes c_1 and c at 24 h after $GdCl_3$ treatment was significantly reduced by 40 and 32%, respectively, which returned to control values at 48 h, without changes in that of cytochromes b and $a+a_3$. It is concluded that $GdCl_3$-induced decrease in liver O_2 consumption is a reversible phenomenon that seems to be due to a diminution in the content of mitochondrial cytochromes c_1 and c.

Key words: Gadolinium chloride; Mitochondrial cytochrome; Oxygen uptake; Kupffer cell functioning; Rat liver

1. Introduction

Kupffer cells are macrophages residing in the sinusoids of the liver, anchored to the endothelial wall and enriched in the periportal areas [1]. Their main functions include presentation of antigens, immunomodulation, phagocytosis, and biochemical attack [2]. The latter feature of stimulated liver macrophages is carried out through the release of several chemical mediators including proteases, cytokines such as interferon-α/β, interleukins 1 and 6, and tumor necrosis factor-α, and free radicals such as superoxide anion and nitric oxide [2]. These mediators are considered essential for the bactericidal and tumoricidal effects of Kupffer cells, and in the toxicity to hepatic endothelial and parenchymal cells [2,3]. Of particular interest is the role that Kupffer cells may play in the hepatotoxicity of a number of xenobiotics [4], galactosamine [5], and endotoxin [6], as well as at hyperthyroid conditions [7]. Blockage of phagocytosis and selective elimination of Kupffer cells are suitable procedures for gaining insight into the functions of liver macrophages in vivo [8], which can be accomplished by means of treatment with compounds such as silica [9], carrageenin [10], ricin [11], methyl palmitate [12], dextran sulfate [4], or gadolinium chloride ($GdCl_3$) [8,13]. Recently, the administration of $GdCl_3$ to rats was found to decrease total rate of O_2 consumption of the liver, measured in perfusion studies [14], an effect that may involve reduced O_2 utilization by microsomal and/or mitochondrial electron transport systems. In this respect, $GdCl_3$ treatment has been shown to diminish the content of total hepatic microsomal cytochrome P450 and the coupled aniline hydroxylase activity by 20–30% [15], in addition to depression of mitochondrial respiration [14]. In fact, isolated liver mitochondria from $GdCl_3$-treated rats exhibit significant decreases in state 3 respiration and in the respiratory control ratio over control values, using glutamate plus malate as electron donors, without changes in ADP/O ratios [14]. Considering that O_2 utilization by the hepatocyte is primarily due to mitochondrial activity over that associated with the microsomal fraction [16], the aim of the current study is to investigate the mechanism underlying the inhibitory effect of $GdCl_3$ on mitochondrial respiration, and the reversibility of the induced changes. To achieve this aim, the influence of $GdCl_3$ on the content of rat liver mitochondrial cytochromes was studied. Results obtained are correlated with changes in the rate of hepatic O_2 uptake and Kupffer cell functioning, assessed in perfusion experiments by means of colloidal carbon infusion.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats weighing 200–250 g were given free access to food and water and maintained on a 12 h light/dark cycle. $GdCl_3$ (10 mg/kg) or saline (controls) was given via tail vein injection, and studies were performed at 0 (controls), 6, 16, 24, and 48 h after treatment under fasting conditions (24 h). All animals used received humane care according to the guidelines outlined in the Guide for the Care and Use of Laboratory Animals by the National Academy of Sciences (National Institutes of Health publication 86-23).

2.2. Mitochondrial studies

Liver mitochondria were isolated according to a standard procedure [17], using a homogenization medium containing 0.25 M sucrose, 0.5 mM EGTA, and 0.1% w/v crystallized and lyophilized bovine serum albumin, pH 7.2. The mitochondrial pellet was washed three times with 0.25 M sucrose and resuspended in this medium to give a concentration of 30 mg of protein/ml [18]. The content of cytochromes c, c_1, b, and $a+a_3$ was measured spectrophotometrically at 25°C with mitochondria (5 mg of protein) treated with cholate/phosphate by using the dithionite-reduced sample versus the oxidized reference [19].

2.3. Liver perfusion, carbon uptake, and carbon-induced respiratory activity

Livers were perfused via the portal vein in a non-recirculating system as described previously [7,14], 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). Livers were

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allowed to equilibrate for 15 min, and perfusate samples were taken every 5 min to measure lactate dehydrogenase (LDH) activity (one unit corresponds to 1 μmol/min at 25°C) [20]. Rates of sinusoidal LDH efflux (U/g liver/min) were calculated from the perfusate activity and the flow rate; at the end of perfusion, liver samples were taken to determine LDH activity in the tissue (U/g liver) [20]. O_2 uptake was continuously determined in the effluent perfusate as it flowed past a Clark-type O_2 electrode, either before (15–30 min perfusion; basal O_2 consumption) or after carbon infusion (30–45 min perfusion).

Uptake of carbon by perfused livers was measured according to Cowper et al. [21], after infusion of 0.5 mg of carbon/ml in the 30–45 min time interval. Rates of carbon uptake (mg/g liver/min) were calculated from the influent minus effluent carbon concentration differences measured spectrophotometrically at 623 nm and the perfusion flow [7,14]. The O_2 consumption induced upon carbon infusion was assessed by the integration of the area under the O_2 uptake curves between 30–45 min and expressed as μmol/g liver [7,14]. This parameter mainly represents the respiratory burst of stimulated Kupffer cells involving NADPH oxidase and nitric oxide synthase activities [2,7]; secondarily, it might involve the mitochondrial respiration of Kupffer cells for energy supply for carbon phagocytosis and the possible action of the eicosanoids, released in this process, on the O_2 uptake of hepatocytes (discussed in [7]). Similar calculations were done for the sinusoidal efflux of LDH, and the results obtained in U/g liver [7,14] were expressed as percentage of the hepatic LDH activity (fractional LDH efflux). These determinations were performed using Krebs-Henseleit bicarbonate buffers without (control rats) or with 10 μg $GdCl_3$/ml ($GdCl_3$-treated rats) to obtain maximal effects, as the in vivo $GdCl_3$ administration does not inactivate all Kupffer cells [8], concentration that does not alter oxidative phosphorylation when added to isolated liver mitochondria [14].

All reagents used were obtained from Sigma Chemical Co. (St. Louis, MO). Results shown are means ± S.E.M. for the number of separate experiments indicated. The statistical significance of differences between mean values was assessed by one-way ANOVA and the Newman-Keuls' test.

3. Results and discussion

Administration of $GdCl_3$ to rats elicited a time-dependent biphasic effect on the basal rate of O_2 consumption of the liver, reaching a maximal decrease (34%) at 24 h after treatment, returning towards control values at 48 h (Fig. 1A). Concomitantly, a significant diminution in Kupffer cell phagocytic activity was observed, evidenced by the decreases in colloidal carbon uptake (Fig. 1B) and in carbon-induced O_2 uptake (Fig. 1C) by perfused livers following 6–24 h of $GdCl_3$ administration. No significant changes in the basal and carbon-induced fractional sinusoidal LDH efflux were observed in these conditions (Fig. 1D), thus pointing out to the adequacy of the design used. At 48 h after $GdCl_3$ treatment, uptake of carbon by perfused livers was recovered to control values (Fig. 1B), whereas carbon-induced O_2 consumption remained lower than control values (Fig. 1C). These findings may be due to the previous observation that $GdCl_3$ leads to an intrahepatic shift in carbon distribution, decreasing the uptake of carbon by liver macrophages and enhancing that of endothelial cells [8]. Although cultured endothelial cells exhibit a respiratory burst in response to several pathological stimuli in vitro (reviewed in [22]), it is uncertain whether they do so in vivo under physiological stimuli or in the intact liver perfused with colloidal carbon. Depression of basal O_2 consumption by perfused livers at 24 h after $GdCl_3$ treatment (Fig. 1A) coincided with a significant diminution in the content of mitochondrial cytochrome c (32%) and c_1 (40%) over control values, without significant changes in that of cytochromes b and $a+a_3$ (Table 1). Cytochrome c_1 is an integral

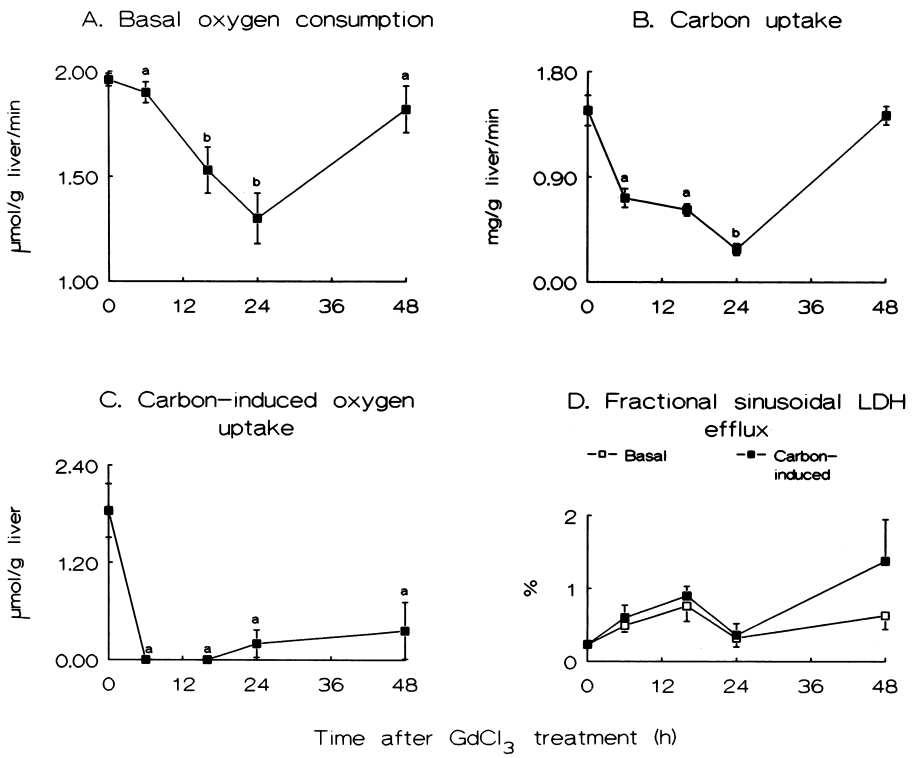

Fig. 1. Time course of the effect of $GdCl_3$ treatment on basal oxygen consumption (A), carbon uptake (B), carbon-induced oxygen uptake (C), and fractional sinusoidal LDH efflux (D) by perfused rat liver. Livers from control rats (time zero) and $GdCl_3$-treated (10 mg/kg) animals were perfused as described in Section 2. Values shown represent means ± S.E.M. for four animals per experimental group. Significance studies: A: a, $P<0.05$ vs. $GdCl_3$ 16 and 24 h; b, $P<0.05$ vs. control, $GdCl_3$ 6 and 48 h. B: a, $P<0.05$ vs. control, $GdCl_3$ 24 and 48 h; b, $P<0.05$ vs. control, $GdCl_3$ 6, 16, and 48 h. C: a, $P<0.05$ vs. control. D: All comparisons are not significantly different.

Table 1
Effect of $GdCl_3$ treatment on the content of rat liver mitochondrial cytochromes

Cytochrome (nmol/mg protein)	Control (a)	$GdCl_3$ (24 h) (b)	$GdCl_3$ (48 h) (c)
c	0.134 ± 0.010 (7)	0.091 ± 0.002 (4)[a,c]	0.125 ± 0.011 (4)[b]
c_1	0.113 ± 0.006 (7)	0.068 ± 0.007 (4)[a,c]	0.124 ± 0.004 (4)[b]
b	0.152 ± 0.010 (9)	0.125 ± 0.008 (5)	0.148 ± 0.013 (5)
$a+a_3$	0.148 ± 0.007 (9)	0.140 ± 0.017 (5)	0.158 ± 0.018 (5)

The content of cytochromes was determined in liver mitochondria isolated from control rats and $GdCl_3$-treated animals (10 mg/kg) at 24 and 48 h after treatment. Values are means ± S.E.M. for the number of animals indicated in parentheses. The statistical significance ($P < 0.05$) is indicated by the letters identifying each experimental group.

membrane protein asymmetrically disposed on the outer surface, whereas cytochrome c is a soluble protein that associates through electrostatic interactions with the outer surface of the mitochondrial inner membrane. Considering that the outer membrane of the mitochondrion is capable of freely admitting molecules of molecular weight of up to 10 kDa [23], interactions between gadolinium, either as free ions or bound to proteins [8], and cytochromes c_1 and c may take place. In agreement with this proposal, eight ion-binding sites have been identified for gadolinium on the surface of cytochrome c, which exhibit different binding specificities [24].

Gd^{3+}-binding to cytochromes c_1 and c may affect the reactivity of redox centers such as iron [25], thus diminishing the content of their functional forms as detected by iron reduction by dithionite (Table 1). In addition, Gd^{3+}-binding to cytochrome c may also affect its reactivity towards cytochrome oxidase, thus interfering with the electron flow and decreasing O_2 consumption, as observed in isolated mitochondria [14] or in perfused livers (Fig. 1A). Alternatively, reduction in cytochromes c_1 and c content may be due to enhanced degradation, as Gd^{3+}-binding to them could represent a marking step making these proteins more susceptible for proteolytic attack, as demonstrated for other post-translational modifications [26], however, further studies are needed to verify this proposal. It is concluded that $GdCl_3$-induced diminution in the basal rate of hepatic O_2 consumption at 24 h after treatment seems to be due to a derangement in mitochondrial O_2 utilization, secondary to reduction in the content of cytochromes c_1 and c, an effect that could also involve depression of microsomal O_2 uptake coupled to reduction in cytochrome P450 content [15]. The reported effects of $GdCl_3$ are reversed at 48 h after its administration (Fig. 1A; Table 1), which might involve synthesis de novo of the cytochromes.

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