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Suppression of peroxisomal lipid β-oxidation enzymes by TNF-α

Konstantin Beier, Alfred Völkl and H. Dariush Fahimi

Institut für Anatomie und Zellbiologie II, Universität Heidelberg, Heidelberg, Germany

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TNF-α is a potent cytokine which induces marked hyperlipidemia. Because of the important role of peroxisomes in lipid metabolism we investigated the effects of human recombinant TNF-α upon rat liver peroxisomal enzymes. Sixteen hours after the administration of a single dose of 25 μg of TNF-α to male rats the activity of peroxisomal fatty acyl-CoA oxidase was reduced by 50%. This was confirmed also by immunoblotting and by quantitative immunoelectron microscopy which in addition revealed substantial reduction of the trifunctional protein (hydratase-dehydrogenase-isomerase) in peroxisomes. These observations suggest that the suppression of peroxisomal β-oxidation may contribute to the perturbation of the lipid metabolism induced by TNF-α.

Peroxisome; Tumor-necrosis-factor; Immunocytochemistry; Lipid β-oxidation; Hyperlipidemia

1. INTRODUCTION

Infection by microorganisms elicits severe hyperlipidemia which is mediated by tumor necrosis factor-α [1]. This potent cytokine which has a wide range of biological activities [2–5] stimulates the hepatic lipogenesis [6,7] while reducing the activity of lipoprotein lipase [8]. Peroxisomes (Po) play a key role in the lipid metabolism (for a review see [9]) and are particularly engaged in the β-oxidation of very long chain fatty acids [10] and unsaturated fatty acids such as eicosanoids [11]. Recently a slight reduction of peroxisomal catalase was reported after long term treatment with TNF but the peroxisomal β-oxidation was not investigated [12]. We have studied the short term effects (16 h) of TNF-α on rat liver Po because at this time the hyperlipidemia reaches its peak [6]. The results show that TNF suppresses markedly the peroxisomal lipid β-oxidation enzymes.

2. MATERIALS AND METHODS

2.1. *Animals*

Male Sprague-Dawley rats (200 g) were supplied by the Medical Research Laboratories of Boehringer Mannheim (Mannheim, Germany) and were kept on a rat chow and water ad libitum. They were injected via the tail vein with 25 or 100 μg of human recombinant TNF-α in 0.5 ml 0.9% saline or saline alone (controls). The TNF was generously provided by BASF/Knoll (Ludwigshafen, Germany). After the administration of TNF, animals were fasted but had free access to water and were sacrificed after 16 h.

Correspondence address: K. Beier, Institut für Anatomie und Zellbiologie II, Im Neuenheimer Feld 307, W-6900 Heidelberg, Germany. Fax: (49) (6221) 564952.

2.2. *Isolation of peroxisomes (Po)*

Rat livers were perfused for 5 min with physiological saline through the portal vein. After homogenization enriched Po fractions were isolated and highly purified Po fractions (>98%) were obtained by Metrizamide-gradient centrifugation as described before [13].

2.3. *Enzyme activity determinations and immunoblotting*

Catalase and acyl-CoA oxidase were determined in liver homogenates using standard methods [13]. For immunoblotting equal amounts of protein from purified peroxisome fractions were subjected to SDS-PAGE and after electrotransfer onto nitrocellulose sheets were incubated with monospecific antibodies to catalase (Cat), acyl-CoA oxidase (AOX) and the trifunctional protein (hydratase-dehydrogenase-isomerase) (PH) as described before [14].

2.4. *Morphometry and quantitative immunoelectron microscopy*

The liver was fixed by perfusion and processed as described [15]. Volume density of peroxisomes was determined in 1 μm light microscopic sections [16] after cytochemical staining of peroxisomes with the alkaline diaminobenzidine-method. For quantitative immunocytochemistry ultrathin sections of LR-white embedded material were labeled with antibodies against Cat, AOX, and PH using the protein A-gold procedure. The labeling density (number of gold particles per μm^2 of peroxisomal matrix) providing information about modulations of peroxisomal enzyme content was determined in electron microscopic sections as described [14,15]. For each enzyme 50 peroxisomes were analyzed from each animal in periportal and the same number in pericentral hepatocytes. For statistical analysis Duncan's multiple range test was used.

3. RESULTS

The animals tolerated the injection of TNF well and did not develop any signs of toxicity.

3.1. *Enzyme activities and immunoblots*

The activity of AOX was reduced significantly ($P < 0.005$) to 50% of the controls, 16 h after treatment with 25 μg TNF (Table I) The catalase activity, on the other hand, was only slightly decreased (being statistically

Table I
Enzyme activities (U/g liver) in controls and TNF-treated animals

	Catalase	Acyl-CoA oxidase
Controls (n=2)	72.9 ± 1.7	300.5 ± 52.5
25 μg TNF (n=6)	57.6 ± 7.4	151.5 ± 11.5

insignificant). The results of immunoblotting (Fig. 1) were in line with enzyme activity measurements showing significant reduction of all three subunits of AOX with only slight reduction of catalase. The peroxisomal trifunctional protein (PH) was also markedly reduced in TNF-treated animals.

3.2. *Morphometry and quantitative immunoelectron microscopy*

The volume density of peroxisomes was not affected at 16 h after TNF-treatment (data not shown) but immunoelectron microscopy revealed significant diminution of gold labeling for peroxisomal β-oxidation enzyme proteins (Fig. 2a,b). Quantitative analysis of immunolabelling (Fig. 3) performed separately on sections from hepatocytes located in the periportal or pericentral regions of the liver lobule revealed no significant alteration for catalase but a dose-dependent reduction of AOX and PH in pericentral hepatocytes. The reduction in periportal hepatocytes for AOX was not statistically significant but reached significance for PH with the 100 μg dose (Fig. 3).

4. DISCUSSION

The present study has revealed that TNF-α suppresses markedly the activity of acyl-CoA oxidase which is the rate limiting enzyme of the peroxisomal β-oxidation spiral [17]. This was confirmed also by immunoblotting and by quantitative immunoelectron microscopy which in addition showed that the peroxisomal trifunctional protein [18] was also markedly reduced in TNF-treated rats. In contrast, the catalase activity was only slightly reduced, although prolonged (5 days) treatment with 100 μg/kg TNF has been reported to lower also the activity of that enzyme in Po [12]. Since one of the distinctive features of Po-lipid β-oxidation is the production of H_2O_2 [17] its down-regulation by TNF should reduce the production of this toxic compound. Indeed, oxygen free radicals have been implicated in TNF-cytotoxicity and there is a striking correlation between the intracellular glutathione level and the resistance of tumors to TNF in vivo [19]. Thus, the reduction in acyl-CoA oxidase activity can also be considered as a 'protective' measure decreasing the possible oxidative stress induced by the generation of H_2O_2. This notion is in line with the reported induction of Mn-superoxide dismutase by TNF which protects the cells against the toxic hydroxyl radicals [20,21], and which is one of the many genes whose transcription is induced by TNF [3,20]. On the other hand TNF down-regulates selectively other genes [20] such as the acyl-CoA synthetase and stearoyl-CoA desaturase-1 which play key roles in the metabolism of long chain fatty acids [22]. Further studies should elucidate the extent of the suppression of other enzymes in peroxisomes not related to lipid β-oxidation. The mechanism of hyperlipidemia induced by TNF is complex but it seems primarily to be due to increased lipogenesis [1,7]. Elevation of

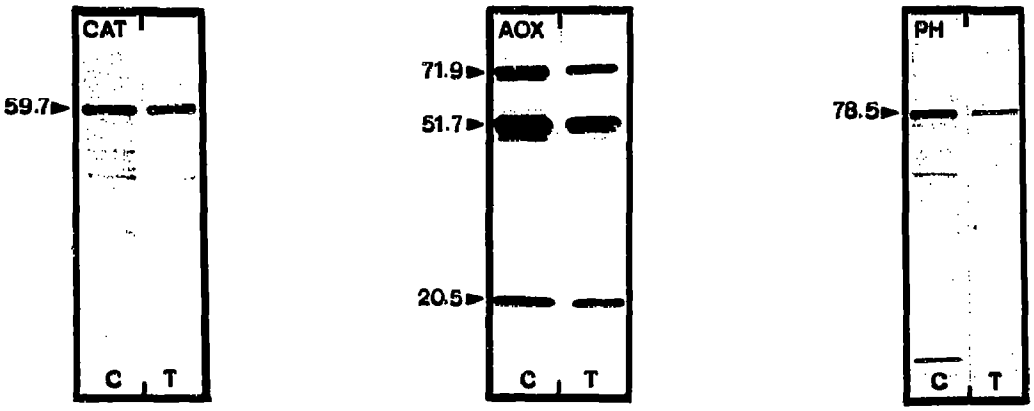

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Fig. 1. Immunoblots of purified Po-fractions from controls (C) and TNF-treated (25 μg) animals (T). The same amount of protein was applied on each lane and blots were incubated with antibodies to catalase (Cat), acyl CoA oxidase (AOX) and trifunctional protein (PH) followed by visualization using the protein A-gold method [14]. The densitometric analysis revealed reduction of Cat by 22%, AOX: subunit A (71.9 kDa) by 54%, subunit B (51.7 kDa) by 35%, subunit C (20.5 kDa) by 59%, and PH by 55%.

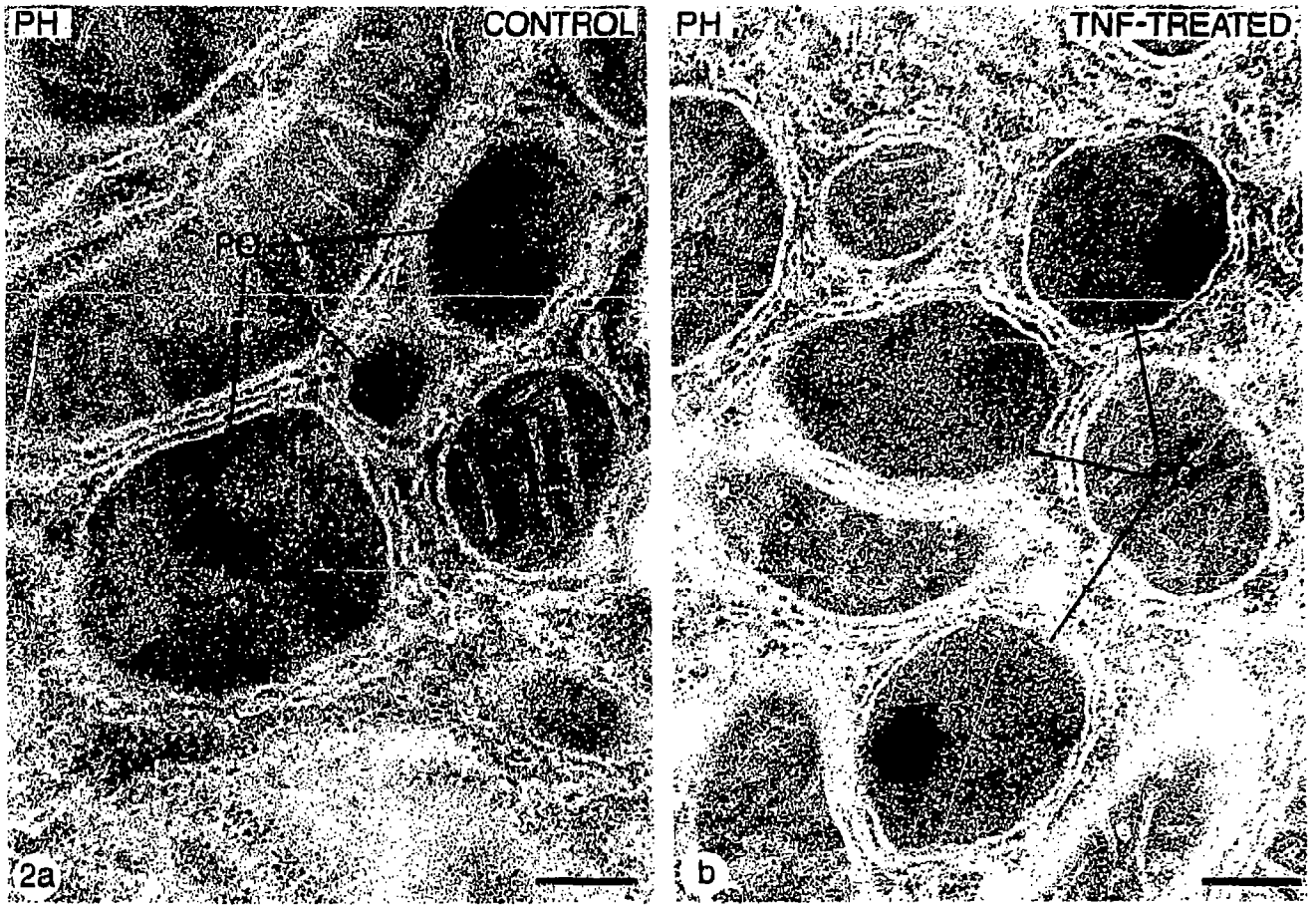

Fig. 2. Electron micrographs showing Po from normal (a) and TNF-treated (b) animals, incubated for immunocytochemical localization of trifunctional protein (PH) by the protein A-gold procedure. Note the marked reduction in labeling of peroxisomes (PO) after TNF treatment. Bars 0.2 μm.

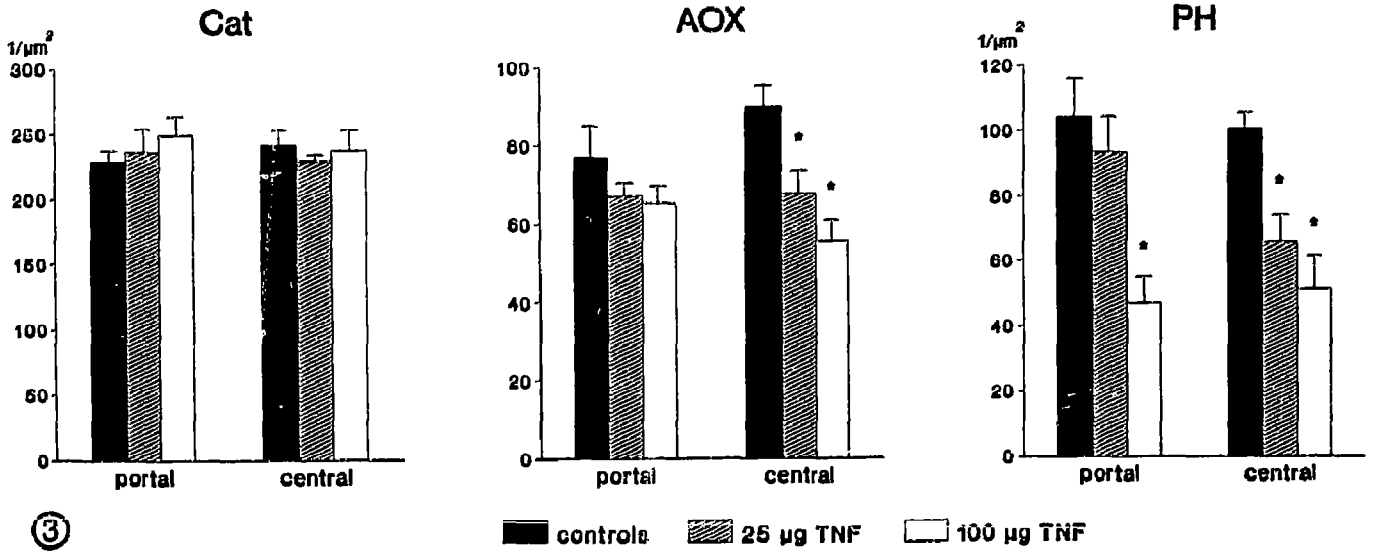

Fig. 3. Labeling density (gold particles/μm^2 Po-matrix) determined in electron micrographs of sections incubated with antibodies against catalase (Cat), acyl-CoA oxidase (AOX), and trifunctional protein (PH). Values are mean ± S.E.M. from n=5 animals per group. *Indicates $P < 0.05$.

citrate, which is an allosteric activator of actyl-CoA carboxylase, has been shown to be primarily responsible for the increase in the activity of this key enzyme of fatty acid biosynthesis [7]. Our observations indicate that, in addition, the reduction of Po-lipid β-oxidation could contribute by TNF-induced hypertriglyceridemia. Peroxisomes preferentially oxidize long-chain fatty acids [10] and polyunsaturated fatty acids such as arachidonic acid [11]. Thus, the reduction in peroxisomal lipid β-oxidation activity could lead to an elevation of arachidonic acid and its metabolites. Those compounds have indeed been implicated in the cytotoxicity of TNF [23]. The pronounced reduction of Po enzymes in pericentral hepatocytes as shown here (Fig. 3) is consistent with the concept of heterogeneity of hepatocytes within the liver lobule [24]. Accordingly the pericentral hepatocytes are primarily engaged in lipid biosynthesis so that our observations would clearly show their preferential involvement in the pathogenesis of TNF-induced hyperlipidemia.

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