Peroxisome Proliferators Activate Kupffer Cells in Vivo

Heidi K. Bojes and Ronald G. Thurman

Laboratory of Hepatology and Toxicology, Department of Pharmacology and Curriculum in Toxicology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599-7365

Abstract

The mechanism by which peroxisome proliferators increase cell replication and cause liver tumors in rodents remains unknown. When activated, Kupffer cells, the resident hepatic macrophages, release a variety of mitogenic stimuli that could theoretically increase cell proliferation in nearby hepatocytes. Therefore, in the present study we evaluated the effect of two potent peroxisome proliferators, nafenopin and WY-14,643, on Kupffer cell activation in vivo. Kupffer cell phagocytosis was determined continuously by monitoring rates of colloidal carbon uptake in the isolated, perfused liver after drug treatment in vivo. In the absence of peroxisome proliferators, colloidal carbon uptake from 88 ± 10 to 110 ± 11 µmol/g/h. Livers from rats treated with either nafenopin (2—24 h) or WY-14,643 (24 h) were perfused for approximately 15 min with Krebs-Henseleit buffer and then with buffer containing colloidal carbon (2 mg/ml). Five h after nafenopin treatment (100 mg/kg, i.g.), basal rates of colloidal carbon uptake of 136 ± 12 mg/g/h were increased to 188 ± 12 and remained elevated after 24 h (203 ± 3 mg/g/h). Nafenopin also increased rates in a dose-dependent manner (one-half maximal response, ~75 mg/kg). Similarly, WY-14,643 elevated rates of colloidal carbon uptake 1.8-fold over controls. Functional parameters of Kupffer cells were also affected. For example, WY-14,643 increased plasma nitrite significantly. This study demonstrates clearly that nafenopin and WY-14,643 activate Kupffer cell phagocytosis, suggesting a role for cell-to-cell communication in the stimulation of cell replication by peroxisome proliferators.

Introduction

Peroxisome proliferators are a group of structurally diverse compounds that include hypolipemic drugs, industrial plasticizers, and halogenated hydrocarbons that cause hepatomegaly, proliferation of hepatic peroxisomes, and induction of several enzymes involved in fatty acid oxidation in the peroxisomal, microsomal, and mitochondrial compartments (1). These chemicals also cause hepatocellular carcinoma in rodents, and the weight of the evidence indicates that they operate via nongenotoxic mechanisms (2). Peroxisome proliferators increase cell proliferation, and considerable data suggest that this phenomena of elevated replication is involved in the mechanism of hepatocarcinogenesis (2, 3). In support of this theory, long-term feeding studies have demonstrated that WY-14,643 is a potent inducer of hepatic tumors, causing sustained elevated rates of cellular proliferation (2).

Marsman et al. showed that WY-14,643 caused only a 2-fold increase in proliferation of primary cultures of hepatocytes, whereas cell replication increased nearly 5-fold in vivo after WY-14,643 treatment (2, 4). Additionally, clofibrate acid, a peroxisome proliferator less mitogenic in vivo than WY-14,643, did not alter cell turnover in vitro (4). Because peroxisome proliferators display weak mitogenic activity in cultured hepatocytes, it is possible that factors from nonparenchymal cells participate in the more potent mitogenic effects observed in vivo. Kupffer cells, the resident hepatic macrophages, are activated by calcium and phagocytosis to release a variety of chemotactic and mitogenic factors and could be involved in growth modulation of nearby hepatocytes. For example, several cytokines (e.g., epidermal growth factor, tumor necrosis factor α, and hepatocyte growth factor) and eicosanoids (e.g., prostaglandin E2), which are produced in the liver almost exclusively by activated Kupffer cells, are direct hepatic mitogens in primary culture systems (5—8). Furthermore, Ankerman et al. (9) showed that antibodies to tumor necrosis factor α inhibited liver regeneration after partial hepatectomy. Moreover, WY-14,643 increased free calcium in cultured Kupffer cells, although high concentrations were used (10). Recently, Rose et al. (11) demonstrated that cultured Kupffer cells engulf the peroxisome proliferator LY171883. They hypothesized that peroxisome proliferators, because of their lipophilic nature, form particles in aqueous media that are taken up by Kupffer cells via phagocytosis. Kupffer cells activated via phagocytosis release various mediators (8, 12).

Carbon particles are selectively taken up by Kupffer cells and rates of colloidal carbon uptake by the perfused liver can be used to monitor phagocytosis, an index of Kupffer cell activation (13). Therefore, the purpose of this study was to determine if two potent peroxisome proliferators, nafenopin and WY-14,643, activate Kupffer cells in the rat in vivo.

Materials and Methods

Animals and Treatment. Male Fisher 344 rats (Charles River Breeding Laboratories) weighing ~150 g were maintained on lab chow and tap water ad libitum and were housed with a 12-h day/12-h night cycle.

Preparation of Colloidal Carbon. A suspension of colloidal carbon was prepared by dialyzing 25—30 ml of India ink (Pelikan black no. 17; Pelikan, Hannover, Germany) against distilled water for 48 h using a Spectrophor semipermeable membrane with a M, 12,000—14,000 exclusion cutoff (Spectrum Medical Industries, Los Angeles, CA). The suspension was stored at 4°C for up to 1 month before use. This stock solution was prepared on the day of the experiment by diluting 6—7 ml into 4 liters of Krebs-Henseleit buffer, yielding A623 of approximately 2.0.

Liver Perfusion. Fed rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and livers were perfused via the portal vein with hemoglobin-free Krebs-Henseleit bicarbonate buffer (pH 7.4; 37°C) saturated with 95% O2-5% CO2 in a nonrecirculating system as described previously (14). Oxygen concentration in the effluent perfusate was monitored continuously with a Teflon-shielded, Clark-type oxygen electrode. Rates of oxygen uptake were calculated from the influent minus effluent concentration differences, the flow rate, and the liver wet weight. A stream-splitter was used to direct a portion of the effluent perfusate through a flow cuvette in an Eppendorf spectrophotometer and A633 due to carbon was monitored continuously (15).

Measurement of Nitrite. Plasma from rats treated with WY-14,643 (100 mg/kg, i.g.) was collected from the descending vena cava. Nitrite was measured colorimetrically with the Griess reaction as accumulated nitrite in plasma (15).
Colloidal carbon uptake varied with length of exposure to nafenopin \((100 \text{ mg/kg i.g.})\) in vivo. As depicted in Fig. 2, basal levels of \(136 \pm 6 \text{ mg/g/h}\) were increased 5 h after nafenopin treatment to \(188 \pm 5 \text{ mg/g/h}\), and values remained elevated after 24 h \((203 \pm 3 \text{ mg/g/h})\). Nafenopin also increased rates of carbon uptake in a dose-dependent manner (one-half-maximal response, \(-75 \text{ mg/kg}\); Fig. 3).

To determine if the activation of colloidal carbon uptake observed with nafenopin occurred with other potent peroxisome proliferators, WY-14,643 was also examined. Fig. 4 shows that WY-14,643 \((100 \text{ mg/kg})\) elevated rates of colloidal carbon uptake by approximately 1.8-fold over control values.

Materials. [4-Chloro-6-(2,3-xylidino)-2-pyrimidinylthio)acetic acid (WY-14,643) was obtained from Chemsyn Science Laboratories (Lenexa, KS). Nafenopin was the generous gift of Ciba-Geigy (Summit, NJ). All other chemicals and reagents were of highest available purity from standard commercial sources.

Statistics. The data were analyzed for statistical significance by one-way ANOVA. Differences between control and treated groups were determined by using the Student-Newman-Keuls statistical method of multiple comparisons.

Results

Colloidal carbon appeared in the effluent perfusate in \(<1\text{ min}\) (Fig. 1B), and the absorbance increased reaching steady state values within 10 min. On termination of carbon infusion, rates returned to basal levels. Within 2–3 min, carbon stimulated oxygen uptake from basal values of around \(88 \pm 10\) to \(101 \pm 11 \text{ mol/g/h}\) (Fig. 1A), but values returned to basal levels after approximately 15 min. Nafenopin and WY-14,643 treatment increased rates of oxygen uptake to values around \(129 \pm 5 \text{ mol/g/h}\). In these livers the addition of carbon did not significantly change oxygen consumption. Livers perfused with colloidal carbon turned intensely black within 10–15 min, and carbon was distributed uniformly across the liver, indicating that the hepatic microcirculation was not altered by infusion of carbon particles.

Discussion

The role Kupffer cells play in phagocytosis of foreign particles is well established, and their ability to release a wide variety of pharmacologically active mediators such as oxygen radicals, cytokines, leukotrienes, and prostaglandins is also well documented (6, 12, 16).
KUPFFER CELLS AND PEROXISOME PROLIFERATORS

Because peroxisome proliferators increase cell proliferation in vivo but elevate replication only weakly in isolated parenchymal cells, the possibility exists that nonparenchymal cells such as Kupffer cells may participate in the mitogenic effect. Indeed, this study demonstrated the activation of Kupffer cells in vivo by two peroxisome proliferators as indexed by increases in phagocytosis of colloidal carbon (Fig. 3). Nafenopin elevated rates of colloidal carbon uptake after in vivo treatment in a manner that was both time- and dose-dependent (Figs. 2 and 3). Similarly, WY-14,643 nearly doubled carbon uptake.

Kupffer cells are activated by a variety of mechanisms, including increased intracellular calcium and phagocytosis (16). Hijioka et al. (10) demonstrated that WY-14,643 elevated intracellular calcium in cultured Kupffer cells. They hypothesized that peroxisome proliferators uncouple mitochondrial oxidative phosphorylation, which decreases cellular ATP and increases intracellular calcium. An increase in calcium is known to activate phospholipase A₂, which breaks down phospholipids to arachidonic acid and ultimately leads to production of prostaglandins and leukotrienes, which have been implicated in growth control (17). For example, Nakagawa et al. (18) demonstrated that arachidonic acid stimulated oxygen uptake in the perfused liver but not in isolated hepatocytes, suggesting that mediators that affect parenchymal cells are released from Kupffer cells. Furthermore, Qu et al. showed that prostaglandin E₂ from Kupffer cells stimulated parenchymal cell oxygen consumption. Furthermore, WY-14,643 stimulated the production of nitric oxide (Results). Taken together, these results are consistent with the hypothesis that activated Kupffer cells influence cellular processes in nearby parenchymal cells. Indeed, this study shows that the addition of carbon increased oxygen uptake, which is largely a parenchymal cell function (Fig. 1).

Peroxisome proliferators most likely activate Kupffer cells via phagocytosis. This group of chemicals forms particles in aqueous media because most of these agents are highly lipophilic. Indeed, phagocytosis of particles of the peroxisome proliferator LY 171883 was recently demonstrated to be both time- and dose-dependent in cultured Kupffer cells (11). It was hypothesized that Kupffer cells are activated by engulfing particles of peroxisome proliferators, leading to the release of mitogenic mediators. It is well established that phagocytosis by macrophages is accompanied by the release of reactive oxygen species and eicosanoids (16). For example, calcium uptake by cultured rat Kupffer cells was stimulated strongly by opsonized zymosan and was accompanied by the release of leukotriene B₄ and superoxide anion (6, 12).

In conclusion, the potent peroxisome proliferators nafenopin and WY-14,643 activated Kupffer cells in vivo, as demonstrated by an increase in phagocytosis of colloidal carbon. These studies support the hypothesis that Kupffer cells are involved in the proliferative mechanism of action of this group of nongenotoxic carcinogens.
KUPFFER CELLS AND PEROXISOME PROLIFERATORS

References


Peroxisome Proliferators Activate Kupffer Cells *in Vivo*

Heidi K. Bojes and Ronald G. Thurman


**Updated version**

Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/1/1

---

**E-mail alerts**

Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.