Agonists of PPAR-α, PPAR-γ, and RXR Inhibit the Formation of Foam Cells from Macrophages in Mice with Inflammation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 11, pp. 556-559, November, 2007
Original article submitted May 31, 2007

We studied the effect of agonists of peroxisome proliferator-activated receptors α and γ and retinoid X receptors on the concentration and synthesis of lipids in macrophages of C57Bl/6 mice with inflammation induced by intraperitoneal injection of zymosan. We revealed a significant increase in [1-^{14}C]oleate incorporation into cholesterol esters and triglycerides, increase in the content of free cholesterol, cholesterol esters, and triglycerides, and formation of oil red-stained lipid inclusions in peritoneal macrophages 24 h after administration of zymosan in a dose of 50 mg/kg. Treatment with agonists of retinoid X receptors and peroxisome proliferator-activated receptors α and γ 30 min before and 12 h after zymosan injection decreased the synthesis of triglycerides and cholesterol esters, reduced the content of free cholesterol, cholesterol esters, and triglycerides in macrophages, and prevented the formation of cytoplasmic lipid inclusions in macrophage-derived foam cells during inflammation.

Key words: *agonists of peroxisome proliferator-activated receptors and retinoid X receptors; macrophages; foam cells; inflammation*

The formation of foam cells from macrophages constitutes a key stage in atherosclerosis, which was considered from the viewpoint of aseptic inflammation in recent years [7]. The formation of foam cells accumulating cytoplasmic lipid vacuoles suggests increased engulfment of modified and native low-density lipoproteins (LDL) and intracellular changes in the synthesis and transport of lipids under the influence of inflammatory factors [15].

Peroxisome proliferator-activated receptors (PPAR) play an important role in the formation of foam cells. PPAR receptors belong to the superfamily of nuclear hormonal receptors and modulate expression of genes involved in the regulation of lipid metabolism and inflammation [12]. PPAR agonists bind to these receptors and stimulate the formation of heterodimers with retinoid X receptors (RXR). Heterodimers of PPAR and RXR bind to promoter or enhancer regions of genes and regulate the expression of target genes. Previous studies showed that administration of PPAR and RXR agonists to LDL receptor knockout mice significantly inhibits the development of atherosclerosis and formation of foam cells [9].

Experiments on mice with experimental aseptic inflammation revealed significantly increased synthesis of triglycerides (TG) and cholesterol esters (CE) [1] and formation of a considerable amount of oil red-stained neutral lipid inclusions in the cytoplasm of peritoneal macrophages 24 h after intraperitoneal injection of zymosan [2].

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0007-4888/07/1445-0713

Here we studied *in vitro* effect of agonists of PPAR-α, PPAR-γ, and RXR on the content and synthesis of CE and TG and formation of oil red-stained lipid inclusions in macrophages.

MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice. The animals were maintained in a vivarium of the Institute of Cytology and Genetics and fed a standard diet. Aseptic inflammation was induced by intraperitoneal injection of zymosan (1 mg/20 g body weight) in 1 ml 0.05 M phosphate buffer with 0.9% NaCl. Control mice received intraperitoneal injection of 1 ml phosphate buffer.

Nuclear hormonal receptor agonists bezafibrate (PPAR-α agonist) [8], rosiglitazone (PPAR-γ agonist) [5], and 9-*cis*-retinoic acid (RXR agonist) [14] were injected intraperitoneally 30 min before administration of zymosan or lipopolysaccharide and 12 h before killing of animals. The doses of these agonists were 10, 10, and 5 mg/kg, respectively. Each group consisted of 15 animals. The measurement of lipid content in macrophages and tumor necrosis factor-α (TNF-α) concentration in the plasma was performed with 12 animals. Morphological study involved 3 animals. The animals were killed by cervical dislocation 24 h after administration of the agonists. The blood was sampled from the retroorbital sinus using a Pasteur pipette, put in Eppendorf tubes, and centrifuged. The plasma was stored at -60°C for 24 h. Peritoneal macrophages were isolated and cultured after blood sampling [1]. [1-^{14}C]Oleate incorporation into CE and TG was studied. The monolayer of cultured macrophages was incubated in RPMI 1640 medium containing 0.2% fatty acid-free bovine serum albumin (BSA) and 5 μl ethanol with 5 μCi [1-^{14}C]oleate in a final concentration of 0.2 mM for 4 h [16]. The cells were washed 3 times with phosphate buffer and 0.2% BSA after incubation. Lipids were extracted with hexane:isopropanol (3:2 v/v) mixture [11]. TG and CE were separated by thin-layer chromatography on Silufol plates [1]. Radioactivity was measured on a Mark-3 liquid scintillation counter (Tracor Analytical) using toluene scintillator. The results were expressed as nmol esterified [1-^{14}C]oleate per mg cell protein. Aliquots of the lipid extract were used for fluorometric study of free cholesterol (FCH) and total cholesterol [6] using standard kits (Cholesterol photometric/fluorometric assay kit, Calbiochem, Cat. N 428901-1KIT). The concentration of cell TG was measured by the colorimetric method with standard kits (Serum triglyceride determination kit, Sigma-Aldrich, Cat. N TR0100). Plasma TNF-α concentration was estimated by solid-phase enzyme immunoassay with ProCon reagents (R&D Systems Inc.) as described elsewhere [3].

Cell protein concentration was measured by the Lowry method [10]. Macrophages were cultured on glass microscopic plates, fixed with 5% paraformaldehyde (15 min), and stained with oil red and hematoxylin [13].

The significance of differences was evaluated by Student's *t* test.

RESULTS

Macrophages were characterized by biochemical and morphological signs of foam cells 24 h after intraperitoneal injection of zymosan, inductor of aseptic inflammation [4]. Zymosan injection increased the synthesis of TG and CSE in macrophages by 19 and 9 times, respectively, compared to the control (Table 1). The content of TG, FCH, and CE in macrophages from animals with inflammation

TABLE 1. Effects of Bezafibrate (PPAR-α Agonist), Rosiglitazone (PPAR-γ Agonist), and 9-*cis*-Retinoic Acid (RXR Agonist) on the Content and Synthesis of CSE and TG and Total Cholesterol Concentration in Mouse Macrophages after Zymosan Administration (*M*±*m*, *n*=12)

Group	Lipid content, μg/mg cell protein			[1-^{14}C]Oleate incorporation into CSE and TG, % of the control	
	total cholesterol	CE	TG	CE	TG
Control	79.1±8.0	10.5±2.1	9.1±1.0	100.0±12.3	100.0±14.5
Zymosan	189±20*	156±14*	257±27*	1186.0±14.8*	1895±295*
Zymosan+bezafibrate	98.5±10.5**	35.9±3.2**	63.4±7.6**	438.0±50.3**	482.0±52.6**
Zymosan+rosiglitazone	91.3±9.0**	48.7±5.4**	96.3±9.8**	286.0±31.2**	729.0±84.2**
Zymosan+9-*cis*-retinoic acid	81.3±8.9**	22.0±2.4**	89.3±9.4**	222.0±24.3**	916.0±98.8**

Note. 100% incorporation of [1-^{14}C]oleate into macrophage CE and TG under control conditions, 7.96 and 30.86 nmol esterified oleate/mg cell protein/4 h, respectively. *p<0.001 compared to the control; **p<0.01 compared to macrophages before zymosan administration.

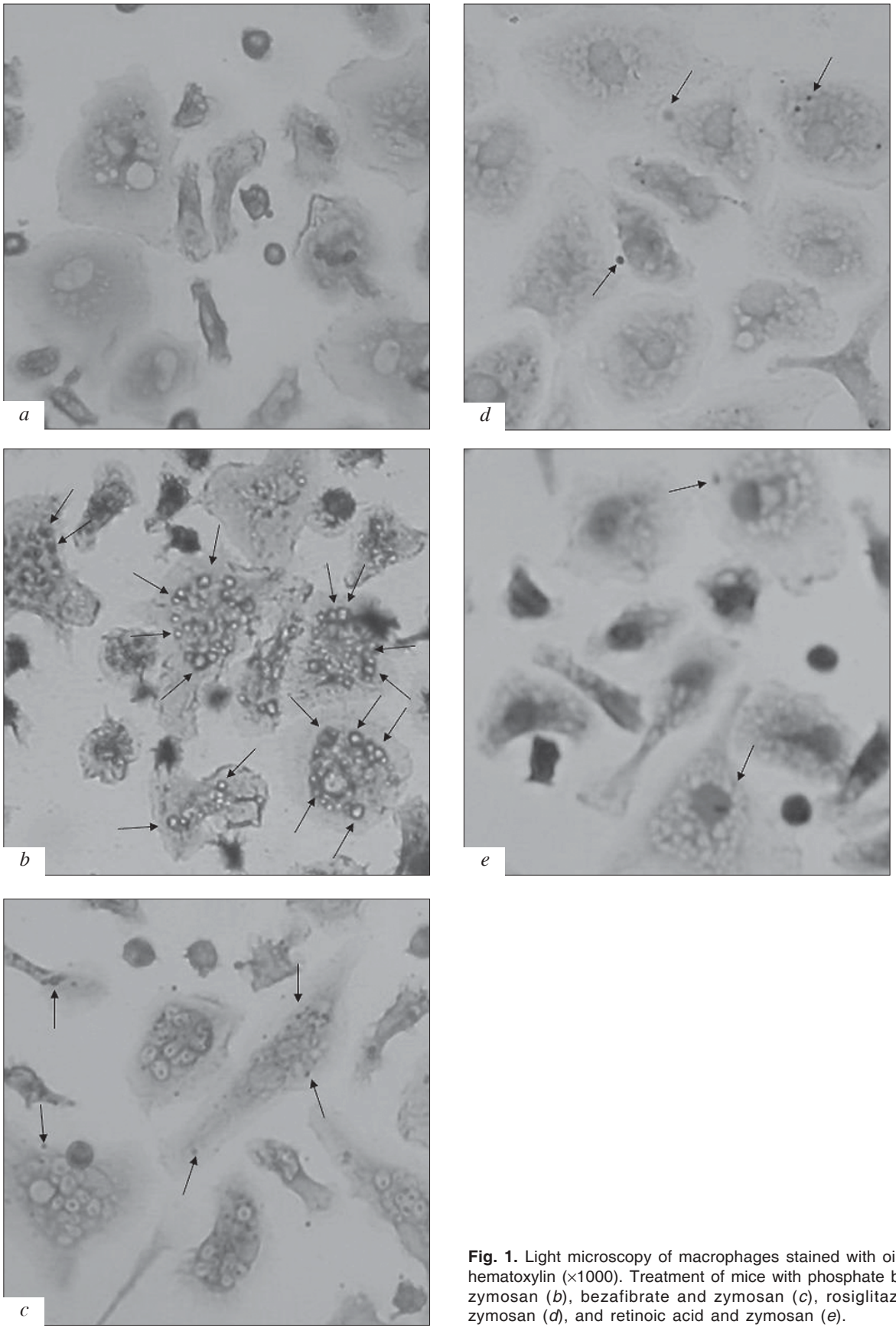

Fig. 1. Light microscopy of macrophages stained with oil red and hematoxylin (×1000). Treatment of mice with phosphate buffer (*a*), zymosan (*b*), bezafibrate and zymosan (*c*), rosiglitazone and zymosan (*d*), and retinoic acid and zymosan (*e*).

increased by 257, 2.4, and 13 times, respectively, compared to the control. Oil red staining revealed massive lipid inclusions in the cytoplasm, which is typical of foam cells (Fig. 1, *a*, *b*). The development of zymosan-induced aseptic inflammation was accompanied by a 7-fold increase in plasma TNF-α concentration (496.0±46.9 *vs*. 72.5±6.8 ng/ml in the control, $p<0.001$).

Administration of bezafibrate, rosiglitazone, and 9-*cis*-retinoic acid prevented the increase in the synthesis and accumulation of lipids in macrophages during zymosan-induced acute inflammatory response. Bezafibrate was most potent in reducing the rate of synthesis and concentration of TG in cells (by 4 times compared to zymosan; and by 1.5 and 2 times compared to rosiglitazone and 9-*cis*-retinoic acid, respectively). Bezafibrate significantly inhibited the synthesis of CE and decreased the content of FCH and CE. Rosiglitazone was more potent than bezafibrate in decelerating the synthesis of CE (by 1.7 times). CE content in macrophages decreased by 3.2 and 4.3 times after administration of rosiglitazone and bezafibrate, respectively. Administration of retinoic acid more significantly inhibited the synthesis of CE (by 2 and 1.3 times compared to bezafibrate and rosiglitazone, respectively) and decreased CE content in cells (by 2 and 1.7 times compared to bezafibrate and rosiglitazone, respectively). Bezafibrate and rosiglitazone decreased plasma TNF-α concentration by 3.4 and 3.6 times, respectively, compared to zymosan. Retinoid acid was 1.5-fold more potent in this respect (496.0±46.9 ng/ml in the zymosan group; 136.2±32.3, 144.7±29.5, and 94.8±6.9 ng/ml in groups of bezafibrate, rosiglitazone, and retinoic acid, respectively; $p<0.01$). Agonists of PPAR-α, PPAR-γ, and RXR significantly decreased lipid accumulation in macrophages, which was manifested in the disappearance of oil red-stained lipid inclusions in the cytoplasm (Fig. 1, *c*-*e*).

PPAR-α agonists are used in clinical practice for the correction of triglyceridemia. PPAR-γ agonists (thiazolidinedione derivatives), including rosiglitazone, are used for the treatment of type 2 diabetes mellitus. Retinoic acid is used for the therapy of epithelial tumors. Our study showed that these agonists of nuclear receptors *in vivo* suppress the formation of foam cells during inflammation. They differ in the ability to inhibit the synthesis and accumulation of lipids in macrophages. We recommend combined treatment with these compounds, which can produce synergistic effect, inhibit transformation of macrophages into foam cells, and prevent the development of atherosclerosis.

This work was supported by the Russian Foundation for Basic Research (grant No. 06-04-48273).

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