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Resveratrol Inhibits Expression and Binding Activity of the Monocyte Chemotactic Protein-1 Receptor, CCR2, on THP-1 Monocytes

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Abstract

Monocyte chemotactic protein-1 and its receptor, CCR2, play a key role in atherosclerosis. We determined the effect of the polyphenol, resveratrol, on CCR2 and the mechanisms involved. Resveratrol treatment inhibited ^{125}I-MCP-1 binding to THP-1 cells; 31%, 56%, 84% decrease for 10, 50 and 100 μM resveratrol, in the absence of any effect on receptor affinity. The inhibitory effect of resveratrol on ^{125}I-MCP-1 binding to THP-1 cells and on CCR2 protein expression determined by FACS analysis was attenuated by treatment with L-NAME (NOS inhibitor), PD98059 (MAPK inhibitor) and LY294002 (PI3K inhibitor), whereas neither X/XO (reactive oxygen species generator) nor ICI182780 (estrogen receptor antagonist) had any effect. Concomitant with a decrease in CCR2 protein expression, resveratrol inhibited THP-1 CCR2 mRNA levels, in the absence of any effect on its stability; 26% and 45% inhibition at 10 and 50 μM resveratrol, respectively. This effect was not altered by co-treatment with L-NAME, PD98059 or ICI182780, but was potentiated by LY294002 and X/XO. Conclusions: Resveratrol inhibits monocyte CCR2 binding activity in an NO-, MAPK- and PI3K-dependent manner, whereas it inhibits CCR2 mRNA in an NO- and MAPK-independent, PI3K-dependent manner. These inhibitory effects of resveratrol on chemokine receptor binding and expression may contribute, in part, to its cardiovascular protective activity *in vivo*.

INTRODUCTION

Epidemiological studies show an inverse relationship between moderate alcohol consumption and cardiovascular disease (1). Moreover, some studies have demonstrated a lower incidence of cardiovascular disease in people who drink red wine, compared to both drinkers of other alcoholic beverages and teetotalers (2). Red wines contain relatively high levels of flavanoids and other polyphenols (3,4). One such polyphenolic phytoalexin is *trans*-3,4′,5-trihydroxystilbene (resveratrol). It is present in *cis* and *trans* isoforms, of which the latter form is believed the biologically active one. In addition to grapes, resveratrol is present in a variety of plant species, including mulberries and peanuts and like other members of the stilbene

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family, is produced in response to pathogen attack, UV-irradiation and exposure to ozone (5). Resveratrol has been widely studied and has been reported to have a variety of potentially anti-atherogenic effects; e.g., it inhibits low density lipoprotein oxidation (4,6), blocks platelet aggregation (7), and activates eNOS (8).

The CC-chemokine, monocyte chemotactic protein-1 (MCP-1) is a potent chemoattractant for monocytes and plays a pivotal role in early atherogenesis by promoting monocyte infiltration to the intima (9). More recently a role for MCP-1 in other cardiovascular diseases such as myocardial ischemia (10) and congestive heart failure (11) has been proposed. MCP-1 is synthesized by endothelial cells and monocytes in response to diverse stimuli including interleukins and oxidized LDL (9) and mediates its effects through activation of the G-protein coupled receptor, CCR2, expressed predominately on monocytes (9). The non-redundant role of CCR2 in monocyte recruitment and atherogenesis has been demonstrated in mice with a targeted disruption of the CCR2 gene. Monocytes from CCR2-deficient mice did not migrate in response to MCP-1 (12). Moreover, CCR2 −/− mice fed a western diet displayed a decrease in lesion size and a decrease in the number of macrophages in these lesions compared to wildtype controls (13).

Here, we examine the effect of resveratrol on CCR2 in THP-1 monocytes. We demonstrate that resveratrol inhibits monocyte CCR2 binding activity, protein and mRNA expression and investigate the signaling mechanisms involved. Our data support the hypothesis that resveratrol is anti-atherogenic, in part, by inhibiting the MCP-1/CCR2 axis.

MATERIALS and METHODS

Resveratrol (3,4,5-trihydroxy-trans-stilbene) was obtained from Sigma (St. Louis, MO). Interleukin-1β was obtained from EMD-Biosciences (San Diego, CA). L-NAME, PD98059, LY294002 and Xanthine/Zanthine oxidase were obtained from Sigma (St. Louis, MO). ICI182780 was obtained from Tocris Bioscience, (Ellisville, MO). All other reagents were of the highest purity commercially available.

THP-1 cells, a human monocytic cell line, were obtained from American Type Culture Collection (ATCC) (Manassas, VA). Growth medium consisted of RPMI-1640, 10% FCS, 0.05 mM 2-mercaptoethanol, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose and 1.5 g/L bicarbonate. To avoid differentiation, cultures were maintained at a density of ~ 2×10^5 cells/ml.

Resveratrol treatment

A stock concentration of 100 mM resveratrol in 50% DMSO was made up fresh each time and diluted in culture medium to the desired concentration. Controls received the same amount of DMSO. THP-1 cells were treated with resveratrol (1–100 μM) for 6 h, unless otherwise indicated. Pharmacological antagonists, when present, were added 30 min before resveratrol.

Cell viability was evaluated by trypan blue exclusion assay and by comparing gross morphology to that of control cells.

^{125}I-MCP-1 binding to THP-1 cells

For binding assays THP-1 cells were washed in PBS before being resuspended in binding buffer (RPMI 1640, 0.5% BSA, 25 mM HEPES, pH 7.4) at 1×10^6 cells/200 μl. For saturation binding analysis, cells were incubated for 90 min at 25°C with 0.02 – 0.4nmol/L ^{125}I-MCP-1 (specific activity, 2200 Ci/mmol) in the absence or presence of 100 nmol/L unlabeled MCP-1. The reaction was terminated by filtration of the reaction mixture through a GF/B filter (presoaked in 0.03% polyethyleneimine) using a Brandel cell harvester (Brandel Biomedical

Research and Development Laboratories, Gaithersburg, MD). Radioactivity on the washed filters was counted in a gamma counter. Specific ^{125}I-MCP-1 binding was determined by subtracting the nonspecific binding estimated in the presence of 100 nmol/L unlabeled MCP-1 from total binding. To determine binding affinity (K_d) and the total number of binding sites (B_{max}), the data underwent Scatchard analysis using Prism software (GraphPad). For 1 point binding assays, THP-1s were incubated with 0.1 nmol/L (~ K_d) ^{125}I-MCP-1, in the absence or presence of 100 nmol/L unlabeled MCP-1.

Western blot analysis

THP-1 cell lysates were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membranes (Hybond-C, Amersham Pharmacia Biotech) by using a Mini Trans-Blot Cell (Bio-Rad) at 80 V for 1h. Equal protein loading in each lane was routinely confirmed by staining membranes with Ponceau S red. Cell lysates (10–20 μg/lane) were analyzed for CCR2 expression using rabbit polyclonal anti-CCR2 antibody obtained from Abcam (Cambridge, MA). MAPK activity was determined by measuring specific phosphorylated pp44ERK-1 and pp42ERK-2 expression; antibodies from Cell Signaling Technologies, Danvers, MA. Activation of eNOS was determined by measuring phosphorylated eNOS using an anti phospho-eNOS (Ser1177) antibody (Cell Signaling Tech., Danvers, MA). PI3-kinase activity was assessed by measuring phosphorylated AKT using an anti phospho-AKT (Thr345) antibody from Upstate Biotechnologies, Lake Placid, NY.

Fluorescence-activated cell sorting (FACS) analysis

THP-1 cells were incubated for 30 minutes on ice with human IgG (MP Biomedicals, Solon, OH) before surface staining with either an anti-human CCR2 monoclonal antibody or a matching isotype control (both labeled with Alexa Fluoro 647 from BD Pharmingen, clones 48607 and 27–35) at 4°C for 20 minutes. Cells were then washed and fixed/permeabilized using 150 uL Cytofix/Cytoperm (BD Pharmingen) for 20 min followed by a 2% FACS Buffer wash. All samples were kept on ice and in dark until analyzed. Cell surface expression of CCR2 was assessed on a Becton Dickinson FACSArray Bioanalyzer System, with 5×10^3 total events collected for each sample. Cells were gated according to light-scatter properties to exclude cellular debris and aggregates. Gating for fluorescence intensity was determined by manually gating in the isotype control THP-1 cell sample, and maintaining that gating for subsequent samples. Data are represented as percent of positive cells and net mean fluorescence intensity (MFI) (as calculated by subtraction of isotype control value from that of the positive antibody).

Northern Blot analysis

Total RNA was isolated from THP-1 cells using TRIzol Reagent (Gibco BRL). Aliquots (10 μg) of the total RNA samples were separated on formaldehyde-agarose gels. The RNAs were transferred and UV crosslinked to nylon membranes and hybridized with ^{32}P-labeled cDNA probes for human CCR2 and GAPDH (ATCC) which were prepared by random priming. Transcripts were quantitated by Kodak ID Image Analysis software (Rochester, NY) and normalized using GAPDH levels for equal loading.

Statistics

The data shown are the mean ± S.E.M. n = number of individual experiments, with a minimum of 3 independent experiments performed. Statistical significance was estimated using the following analysis: Unpaired Student's t-test for comparison of two groups; Wilcoxon signed rank test for the densitometric data; ANOVA was used for multiple comparisons. A value of $P<0.05$ was considered significant.

RESULTS

Resveratrol and CCR2 receptor binding

CCR2 receptor surface expression on whole THP-1 cells was determined by ^{125}I-MCP-1 saturation binding analysis. ^{125}I-MCP-1 bound to control THP-1 cells with an equilibrium binding constant (K_d) = 0.125±0.01 nM and maximal binding sites (B_{max}) = 3.52±0.42 fmol/10^6 cells (14). Non-specific binding was ~ 5–20% of total binding. CCR2 binding characteristics were similar in THP-1 cells and in freshly isolated peripheral blood monocytes (data not shown). Resveratrol treatment dose-dependently inhibited ^{125}I-MCP-1 binding to THP-1 cells; 31%, 56% and 84% decrease for 10, 50 and 100 μM resveratrol, respectively (Figure 1). In contrast, treatment with ethanol (100 mM) had no significant effect on ^{125}I-MCP-1 binding to THP-1 cells (Figure 1). Competitive displacement binding analysis suggested that the resveratrol-induced decrease in the maximum number of binding sites in THP-1 monocytes (Figure 2a) was not associated with any change in the affinity of MCP-1 for its CCR2 receptor in these cells (Figure 2b); the B_{max} and K_d for Resveratrol (50 μM) treated THP-1 cells was 1.44±0.21 fmol/10^6 cells (n=3, $p<0.05$ vs control) and 0.095±0.02 nM, respectively.

Resveratrol stimulated MAPK, eNOS and PI3-kinase activity in THP-1 cells (Figure 3). As determined by Quantikine iNOS immunoassay, resveratrol had no significant effect on LPS/TNF-stimulated THP-1 iNOS (data not shown). The inhibition by resveratrol of ^{125}I-MCP-1 binding to THP-1 cells was prevented by co-treatment with the MAPK inhibitor, PD98059 (10 μM), and the nitric oxide synthase (NOS) inhibitor, L-NAME (100 μM) (Figure 4). Furthermore, the PI3 kinase inhibitor, LY294002 (10 μM), attenuated the resveratrol response (Figure 4). Treatment with the reactive oxygen intermediate generating system, xanthine/xanthine oxidase (500 μM/10 milliunits/ml), or with the estrogen receptor antagonist, ICI 182780 (10 μM), did not alter the inhibitory effect of resveratrol on ^{125}I-MCP-1 binding to THP-1 cells (Figure 4). Treatment with any of these inhibitors alone did not significantly affect ^{125}I-MCP-1 binding to THP-1 cells (data not shown). Similarly, FACS analysis showed that the resveratrol inhibition of CCR2 protein expression was attenuated by PD98059, L-NAME and LY294002, whereas xanthine/xanthine oxidase and ICI 182780 had no effect (Figure 5).

Effect of resveratrol on CCR2 protein expression and mRNA levels

THP-1 CCR2 protein levels, as determined by Western blot analysis, were inhibited in a time- and dose-dependent manner by resveratrol (Figure 6). Similar results were obtained by FACS analysis of surface CCR2 protein expression (data not shown). Total RNA was isolated from THP-1 monocytes and used for the analysis of CCR2 mRNA by Northern blot. Resveratrol treatment dose-dependently inhibited THP-1 CCR2 mRNA expression; 12±5, 50±5 and 70 ±10% decrease for 10, 50 and 100 μM, respectively (Figure 7a).

Resveratrol and CCR2 mRNA stability

CCR2 mRNA expression can be regulated at the transcriptional or post-transcriptional level. To study whether the inhibitory effect of resveratrol on CCR2 gene expression is caused by a decrease in mRNA stability, THP-1 cells were treated with or without resveratrol (50 μM) followed by incubation with actinomycin D (10 μg/ml), to prevent further synthesis of CCR2 mRNA. Total RNA was extracted at different times. As indicated in Figure 7b, resveratrol had no significant effect on CCR2 mRNA degradation rate in actinomycin D-treated cells; estimated half-life of the CCR2 transcript was ~ 1.5 - 2h in both control and resveratrol treated cells. These data indicate that the inhibitory effect of resveratrol on CCR2 was not caused by decreased stability of CCR2 mRNA.

To investigate the signaling mechanisms mediating the inhibitory effect of resveratrol on CCR2 mRNA expression, THP-1 cells were preincubated with the various antagonists before resveratrol exposure. Treatment with L-NAME, PD98059 or ICI182780 alone had no effect on CCR2 mRNA levels (Figure 8a). Moreover, pretreatment with these antagonists did not significantly affect the resveratrol CCR2 mRNA inhibitory response (Figure 8b). In contrast, treatment with LY294002, alone, significantly inhibited CCR2 mRNA and it potentiated the resveratrol inhibitory response (Figure 8). Similarly, treatment with X/XO alone inhibited CCR2 mRNA and it also potentiated the resveratrol inhibitory response (Figure 8).

DISCUSSION

The main findings of the present study are that resveratrol, a polyphenol found in red wine and peanuts, inhibits CCR2 binding activity and expression in monocytes. In particular, resveratrol inhibited CCR2 protein expression and the binding of ^{125}I-MCP-1 to CCR2 on THP-1 monocytes in an NO-, MAPK- and PI3K-dependent manner. Moreover, resveratrol decreased CCR2 mRNA expression in an NO- and MAPK-independent, PI3K-dependent manner. These *in vitro* findings support a novel effect of resveratrol at the level of a chemokine receptor that may be relevant to its putative cardioprotective capacity *in vivo*.

Much evidence exists supporting a central role for the MCP-1/CCR2 axis in the pathogenesis of atherosclerosis. In response to several atherogenic stimulants such as oxidized LDL, CD40 ligand, platelet derived growth factor and interleukin-1β, MCP-1 is induced in endothelial cells, smooth muscle cells and monocytes and promotes the transmigration of monocytes through the endothelial barrier (9). The role of MCP-1 in human disease has been implicated by immunohistochemical studies of atherosclerotic plaques (15). Analysis of atherosclerotic lesions in apo-E mice overexpressing a murine MCP-1 transgene, when compared with the response of control apo-E knockout mice, revealed enhanced lipid staining, increased oxidized lipid content and increased immunostaining for macrophage cell surface markers (16). CCR2 knockout mice, crossed with apo-E knockout mice and fed a western diet displayed decreased lesion size and macrophage content in these lesions (13). Several agents, including homocysteine and oxidized LDL, have been shown to affect CCR2 expression (17,18). Interestingly, CCR2 expression is significantly increased in circulating monocytes in hypercholesterolemic humans (18). Moreover, Ishibashi M et al., demonstrated that CCR2 played a critical role in vascular inflammation and angiotensin II-induced hypertension (19).

There is also growing evidence that MCP-1/CCR2 may play an important pathogenic role in other cardiovascular diseases such as myocardial ischemia (10,20) and congestive heart failure (11). Hayasaki et al., reported in mice that CCR2 deficiency protected the myocardium after myocardial ischemia-reperfusion injury (20). Moreover, Furukawa et al., reported that neutralization of MCP-1 before, and immediately after arterial injury may be effective in preventing restenosis after angioplasty (21). These studies provide compelling evidence for a direct role of CCR2 in cardiovascular disease. Therefore, agents, acting by decreasing CCR2 receptor expression and/or ligand binding/signaling, potentially represent innovative pharmacological tools for the prevention of cardiovascular-related diseases.

There was a significant decrease in ^{125}I-MCP-1 binding in resveratrol treated THP-1 cells as compared to controls in the absence of any change in the affinity of MCP-1 for its receptor. Pretreatment of THP-1 cells with PD98059 and L-NAME completely blocked, whereas pretreatment with LY294002 attenuated, the resveratrol-induced decrease in ^{125}I-MCP-1 binding, implicating MAPK, NO and PI3K, respectively, in the response. Alteration of these pathways by resveratrol has been reported previously but a link between these effects and modulation of CCR2 in monocytes had not been established. Resveratrol has been reported to both stimulate (22,23) and inhibit (24) MAPK in various cell types. Of interest, Klinge et al.,

demonstrated that resveratrol increased ERK1/2 phosphorylation in BAEC, an effect that was, in part, estrogen receptor dependent (22). While a review of the literature revealed no other reports of the effect of resveratrol on monocyte MAPK, we found that resveratrol stimulated MAPK in THP-1 cells as evidenced by phosphorylation of ERK1/2. As regards NO, resveratrol has been previously reported to activate NOS in vascular endothelial cells (8) and induce NO release from intact vessels (25). In our hands, resveratrol stimulated eNOS and AKT phosphorylation in THP-1 cells, in the absence of any effect on cytokine-stimulated iNOS. We found that resveratrol stimulated ERK and Akt phosphorylation to a similar extent. However, LY294002 attenuated, more modestly than PD98059, the resveratrol effect on CCR2 binding. While the precise mechanisms are unknown, this discrepancy is likely explained by the fact that phosphorylated Akt is a regulator of multiple downstream events, not all of which may be involved in mediating the resveratrol-induced decrease in CCR2 binding. Our data shows that treatment with an inhibitor of PI3K reduced THP-1 CCR2 mRNA expression, in the absence of any effect on CCR2 binding activity. A link between the PI3K pathway and CCR2 expression has been hinted at previously. In patients with diabetes, a condition associated with decreased PI3K activity (26,27), monocyte CCR2 surface expression determined by FACS was modestly increased compared to controls (28). However, given the many variables between them, our study and the latter one are difficult to compare. In addition, it has been established that activation of CCR2 by MCP-1 results in PI3K activation (29,30). Taken together, these data suggest that further investigation into the role of the PI3K/Akt pathway in regulating CCR2 mRNA, surface expression and binding activity is warranted. Of interest, the inhibitory effect of resveratrol on CCR2 protein expression and binding activity was MAPK- and NO-dependent, whereas its inhibitory effect on CCR2 mRNA was MAPK- and NO-independent, suggesting divergent signaling pathways. Of note, our data are supported by a recent study that demonstrated a decrease in MCP-1 and CCR2 mRNA levels in the aortas of ApoE knockout mice fed a diet supplemented with a mixture of pure polyphenols (cathechin, caffeic acid and resveratrol) (31).

A structural similarity exists between resveratrol and the synthetic estrogen diethylstilbestrol and it has been postulated that the estrogen-like phytoestrogenic properties of resveratrol could be the basis of its putative cardioprotective effect. Resveratrol can both activate and inhibit estrogen receptors (22,32), and estrogen receptors are expressed on monocytes and macrophages (33). To address this possibility, we conducted our experiments in the absence or presence of the estrogen receptor antagonist ICI 182780. However, our data suggest that neither the resveratrol effect on CCR2 binding activity nor on mRNA expression was ER-dependent.

Resveratrol is an antioxidant with the ability to scavenge reactive oxygen species (34). Saccani et al., reported that the redox status is a crucial determinant in the regulation of chemokine receptors (35). Specifically, they reported that the antioxidant PDTC inhibited CCR2 mRNA expression by decreasing transcript stability (35). We investigated whether oxidative signals might counteract the inhibitory action of resveratrol on CCR2 but found that the reactive oxygen intermediate generating system, xanthine/xanthine oxidase (X/XO), had no effect on resveratrol's inhibition of CCR2 protein expression or ^{125}I-MCP-1 binding to CCR2. However, with respect to CCR2 mRNA levels, X/XO itself decreased CCR2 mRNA and further potentiated the resveratrol inhibitory response. These data suggest that while the redox state appears to be important in regulating CCR2, at least at a mRNA level, that the resveratrol effect on CCR2 appears independent of its antioxidant potential.

The concentrations of resveratrol used in our study ranged from 1–100 μM. There were significant effects of resveratrol on CCR2 at concentrations as low as 10 μM. While these levels are likely higher than serum levels achieved with moderate red wine consumption in humans, estimated in the nM range, (36), they are pharmacologically relevant and in line with

serum levels achieved in animal studies (μM range). Although moderate drinking of any type of alcoholic beverage helps reduce the incidence of coronary artery disease (2), there is some evidence that red wine confers an additional benefit. Of interest, we have previously reported that both resveratrol and ethanol inhibit MCP-1 synthesis, the latter in the absence of any effect on CCR2 (37,14). Taken together with our current study, these data support synergistic effects of ethanol and resveratrol, both found in red wine, on the MCP-1/CCR2 axis and might explain the greater beneficial effect of red wine over other alcoholic beverages.

Despite the inverse relationship between moderate red wine consumption and the risk of coronary heart disease and the evidence for a prominent role of CCR2 in the pathogenesis of cardiovascular disease, few studies have investigated the effect of resveratrol on CCR2 expression and/or binding activity. Our data demonstrate a dual inhibitory effect of resveratrol on CCR2 expression and binding activity and support modulation of CCR2 as a potential novel cardioprotective mechanism of resveratrol.

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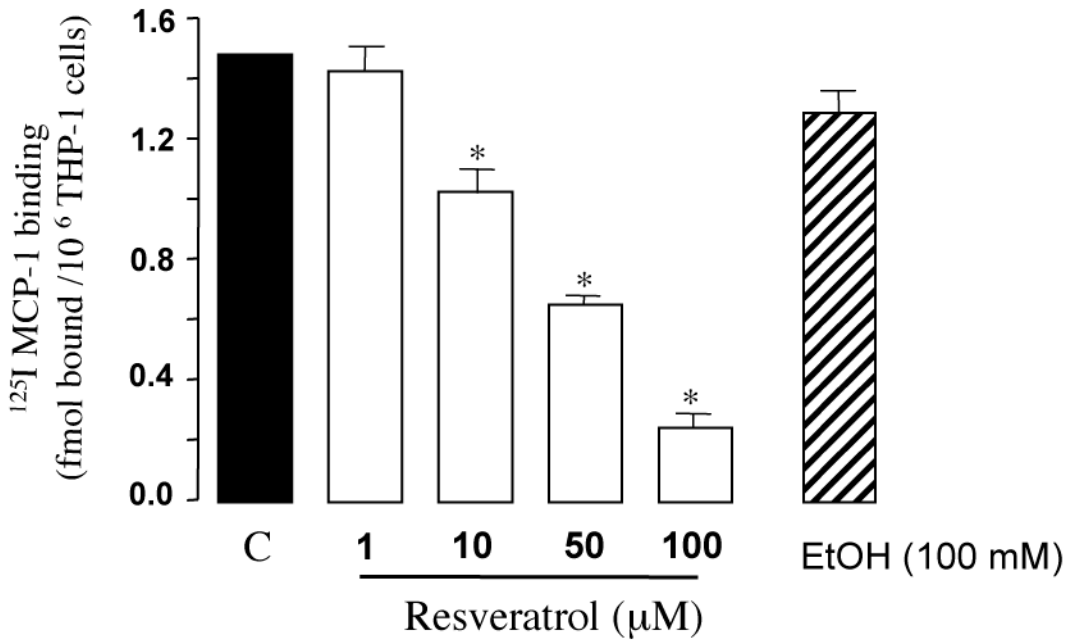

Figure 1.

Effect of resveratrol on ^{125}I-MCP-1 binding to THP-1 monocytes. Binding of ^{125}I-MCP-1 was determined by equilibrium binding assay as described in Methods. THP-1 cells were treated (6h) with or without Resveratrol (1–100 μM) or ethanol (100 mM) before binding was measured. For these 1 point binding assays, ^{125}I-MCP-1 was added at a concentration of 0.1 nM (~Kd) in the absence or presence of 100 nM MCP-1 to determine non-specific binding. Data are means ± SEM; n=3. *P<0.05 vs. control.

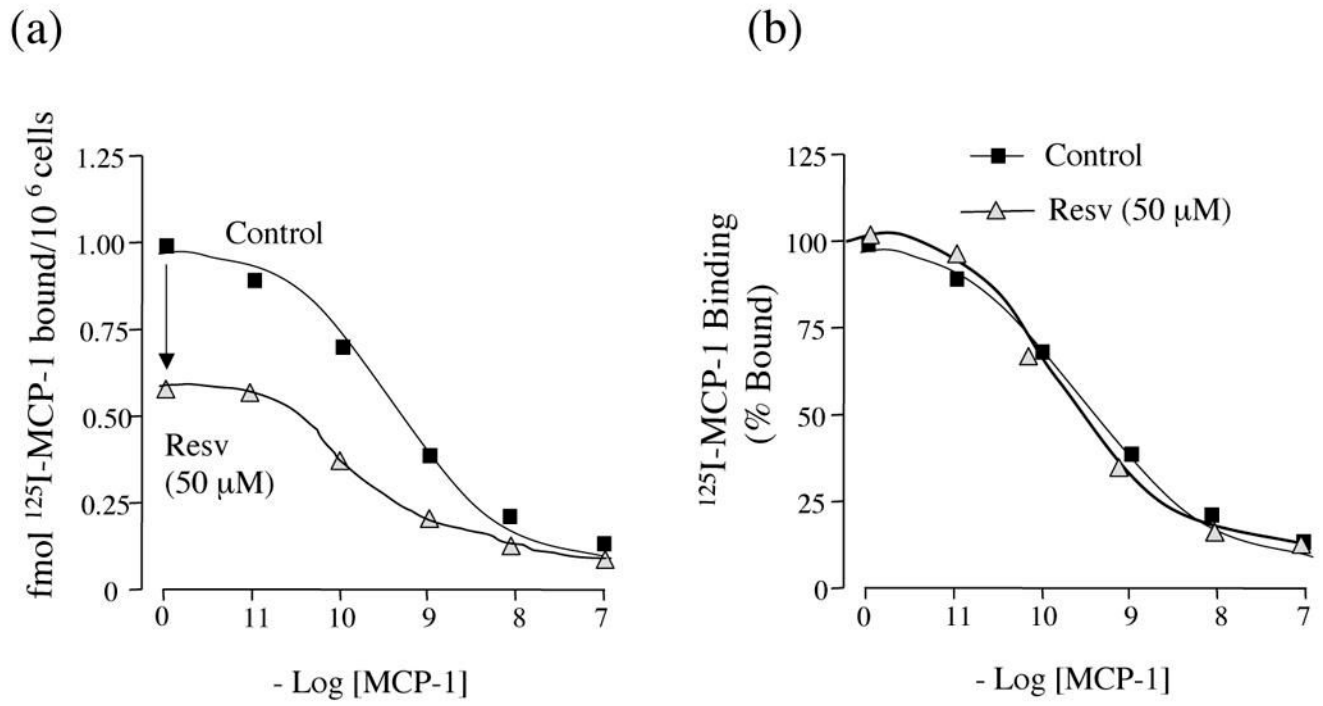

Figure 2.

(a) Displacement of ^{125}I-MCP-1 binding by unlabeled MCP-1 in control and resveratrol (50 μM, 6h) treated THP-1 cells. (b) Data expressed as % Bound. Representative experiment shown, mean of duplicate determinations.

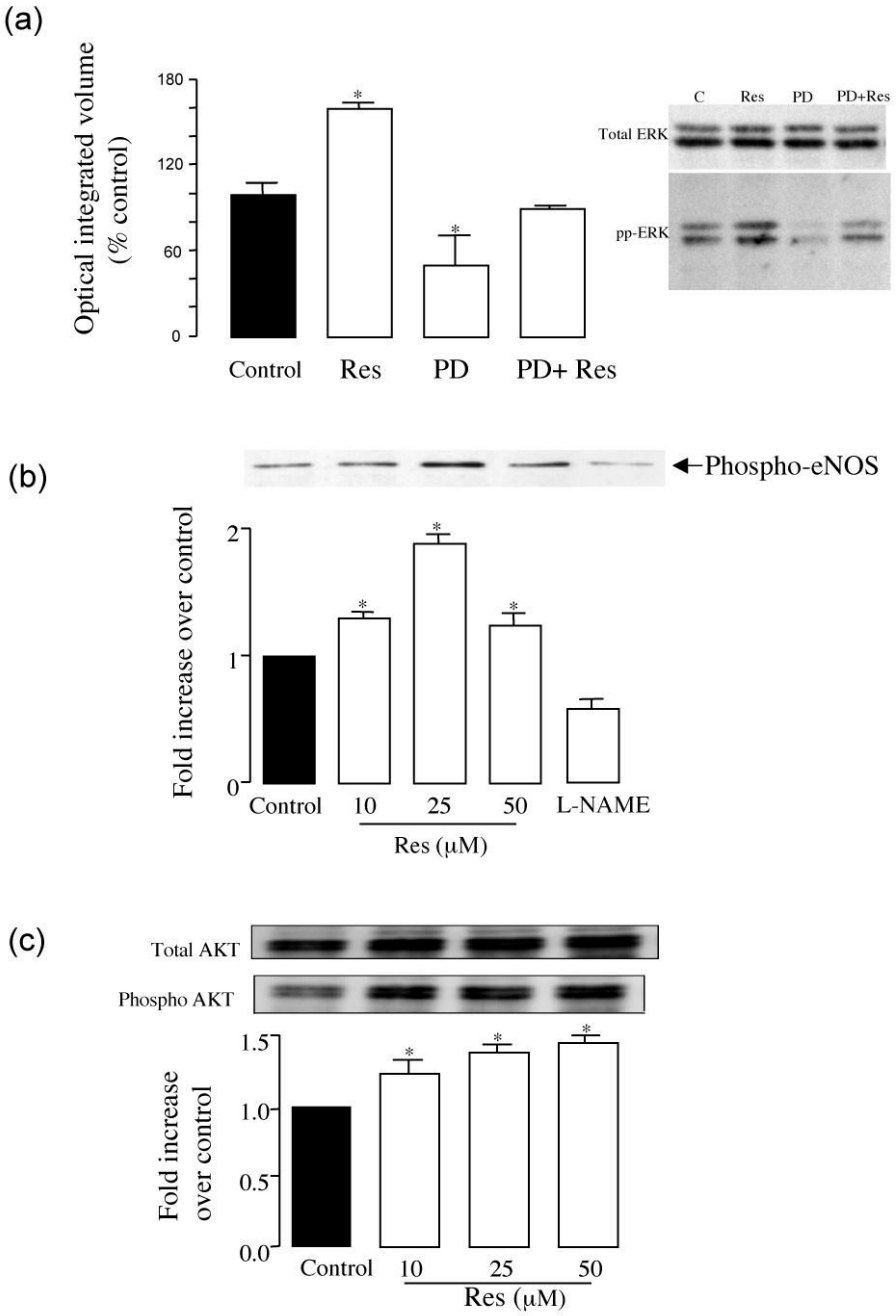

Figure 3.

THP-1 monocytes were preincubated for 30 min with the indicated pharmacological antagonists before treatment with or without resveratrol for 6h. Western blot analysis was performed on cell lysates and (a) MAPK activity determined by measuring specific phosphorylated pp44ERK-1 and pp42ERK-2 expression, (b) activation of eNOS determined by measuring phosphorylated eNOS, and (c) PI3K activity was assessed by measuring phosphorylated AKT (phospho AKT). Res = resveratrol (50 μM, unless otherwise indicated), PD=10 μM PD98059, L-NAME (100 μM). Representative Western blots are shown together with the cumulative densitometric data of 3 separate experiments. Data are means ± SEM. *P<0.05 vs control.

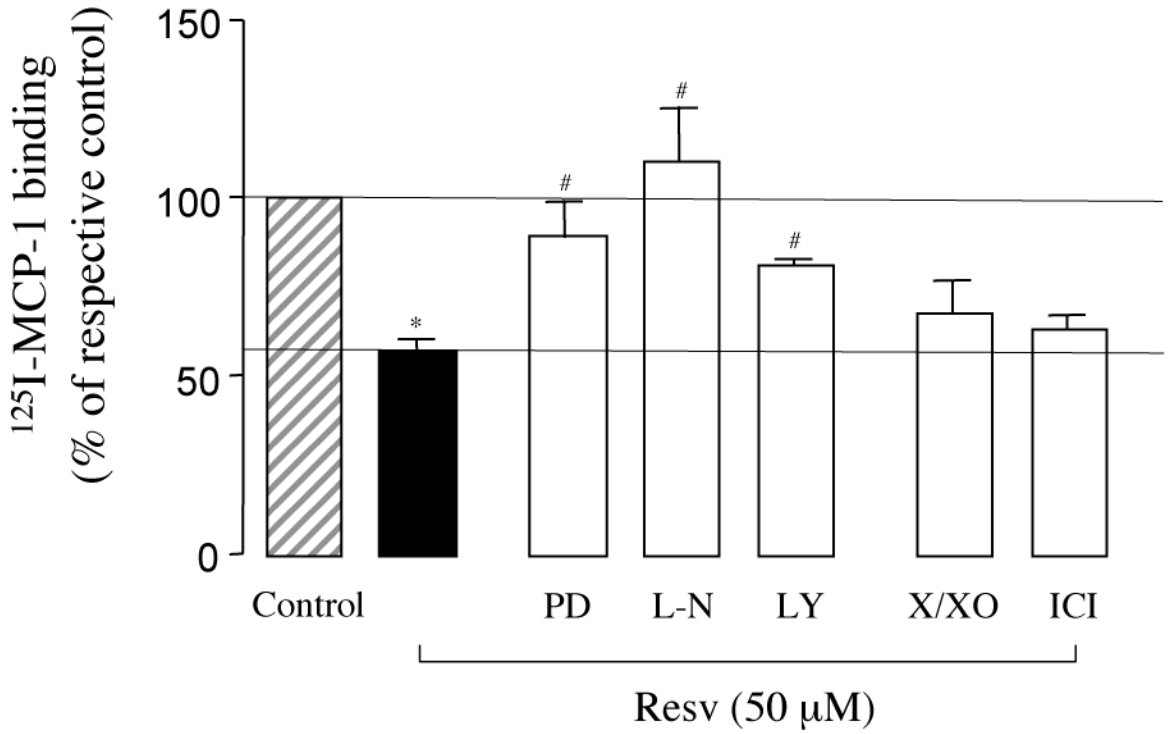

Figure 4.

THP-1 monocytes were preincubated for 30 min with the indicated pharmacological antagonists before treatment with or without resveratrol for 6h. Binding of ^{125}I-MCP-1 was then determined as described in Methods. PD=10 μM PD98059; L–N=100 μM L-NAME; LY=10 μM LY294002; X/XO=500 mM//10 miliunits/ml xanthine/xanthine oxidase; ICI=10 μM ICI182780. Data are means ± SEM, n=4–5. *P<0.05 vs control; #P<0.05 vs resveratrol alone.

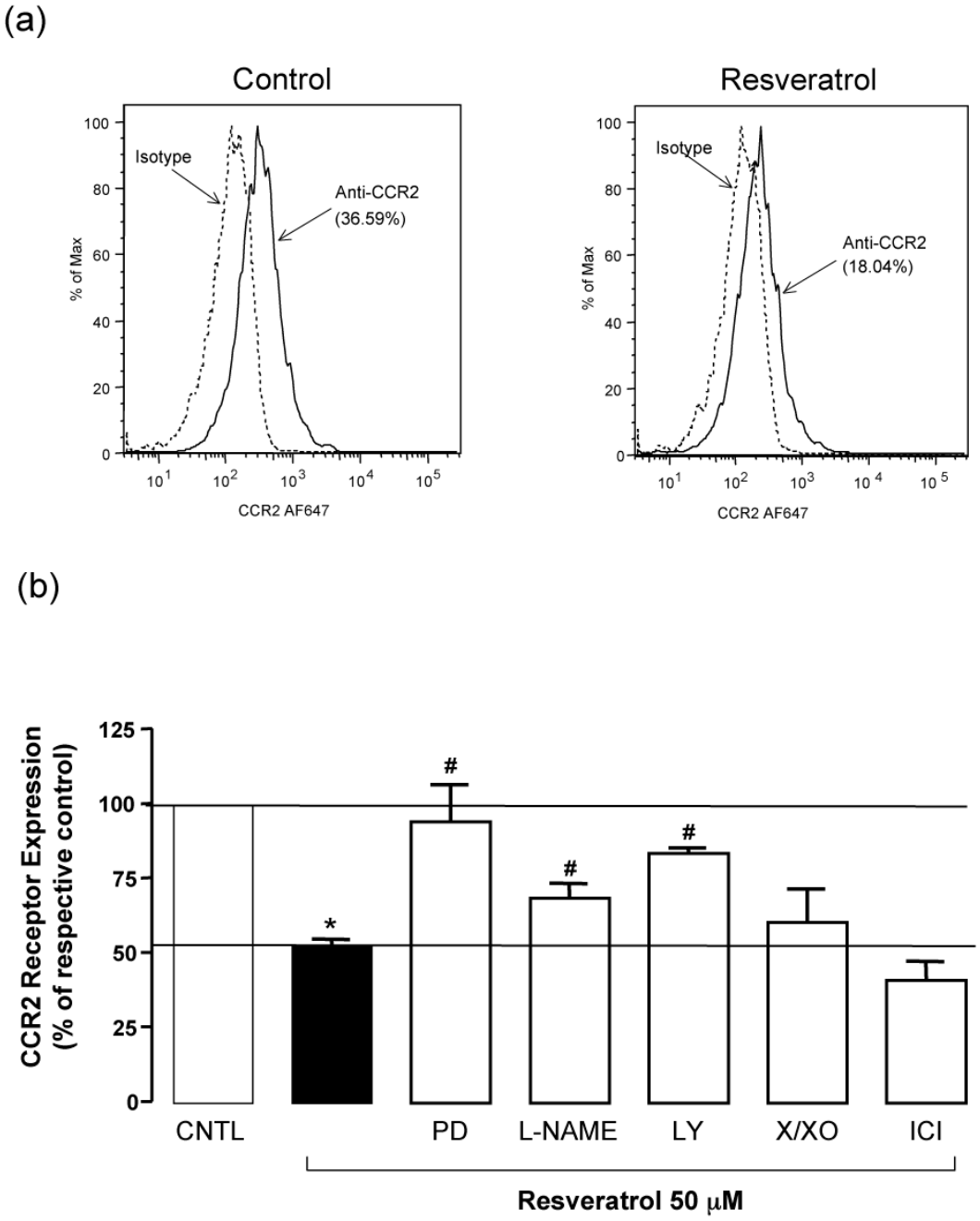

Figure 5.

(a) FACS analysis of control (DMSO) and resveratrol (50 μM) treated THP-1 cells using anti-CCR2 antibody (solid line) or isotype control antibody (dashed line). (b) THP-1 monocytes were preincubated for 30 min with the indicated pharmacological antagonists before treatment with or without resveratrol (50 μM) for 6h. The effect of resveratrol ± the inhibitors on surface CCR2 receptor expression was then determined by FACS analysis. Data are mean ± SEM; n=3–4. *p<0.05 vs control, #p<0.05 vs resveratrol alone.

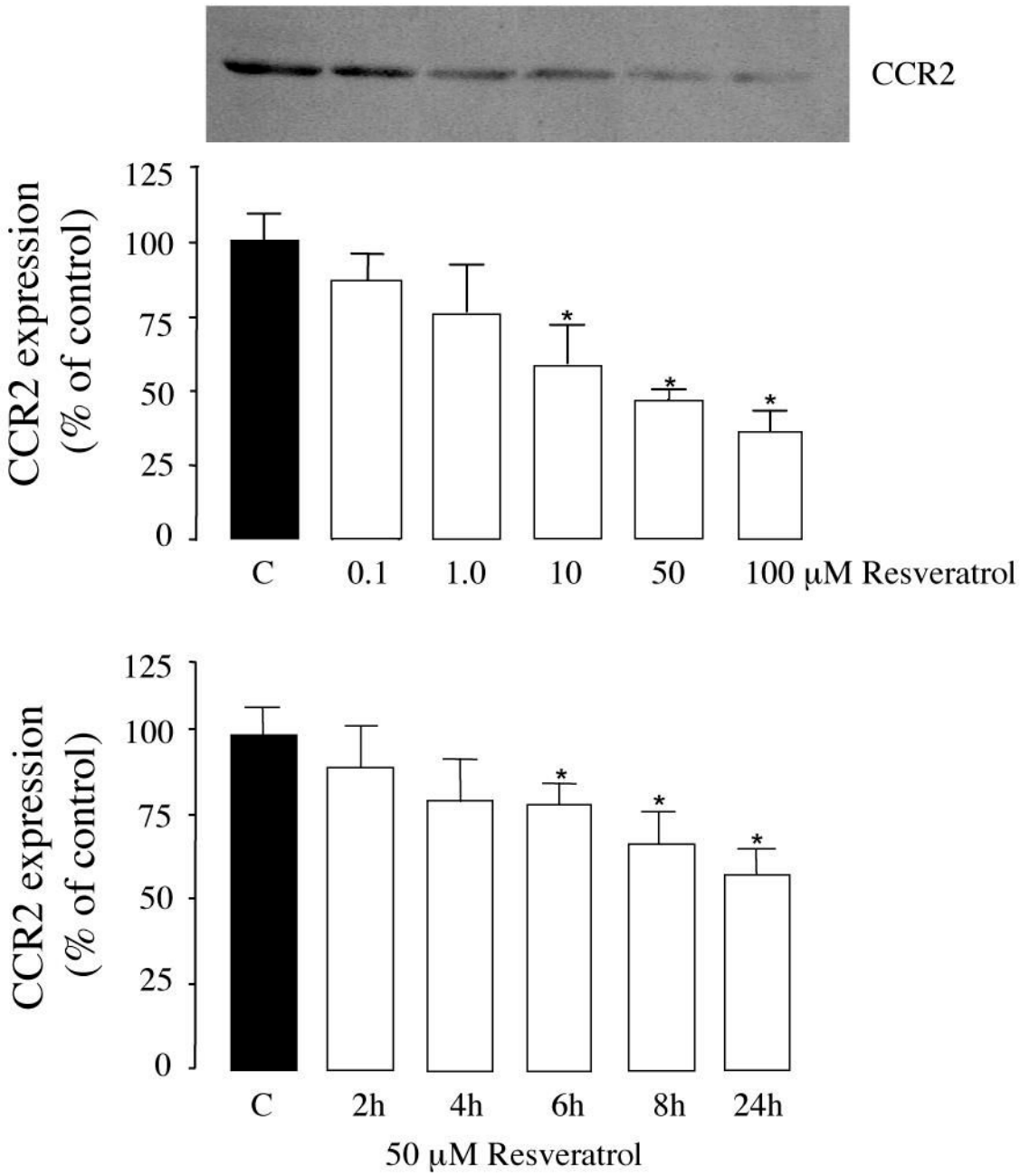

Figure 6.

Effect of Resveratrol on CCR2 protein expression; dose-response and time course. THP-1 cells were treated with or without resveratrol (0.1 – 100 μM) for 24h (top), or with 50 μM resveratrol for different times (2–24h) (bottom). CCR2 protein expression was determined in cell lysates by Western blot analysis. Data are mean ± SEM, n=3–4. *P<0.05 vs control.

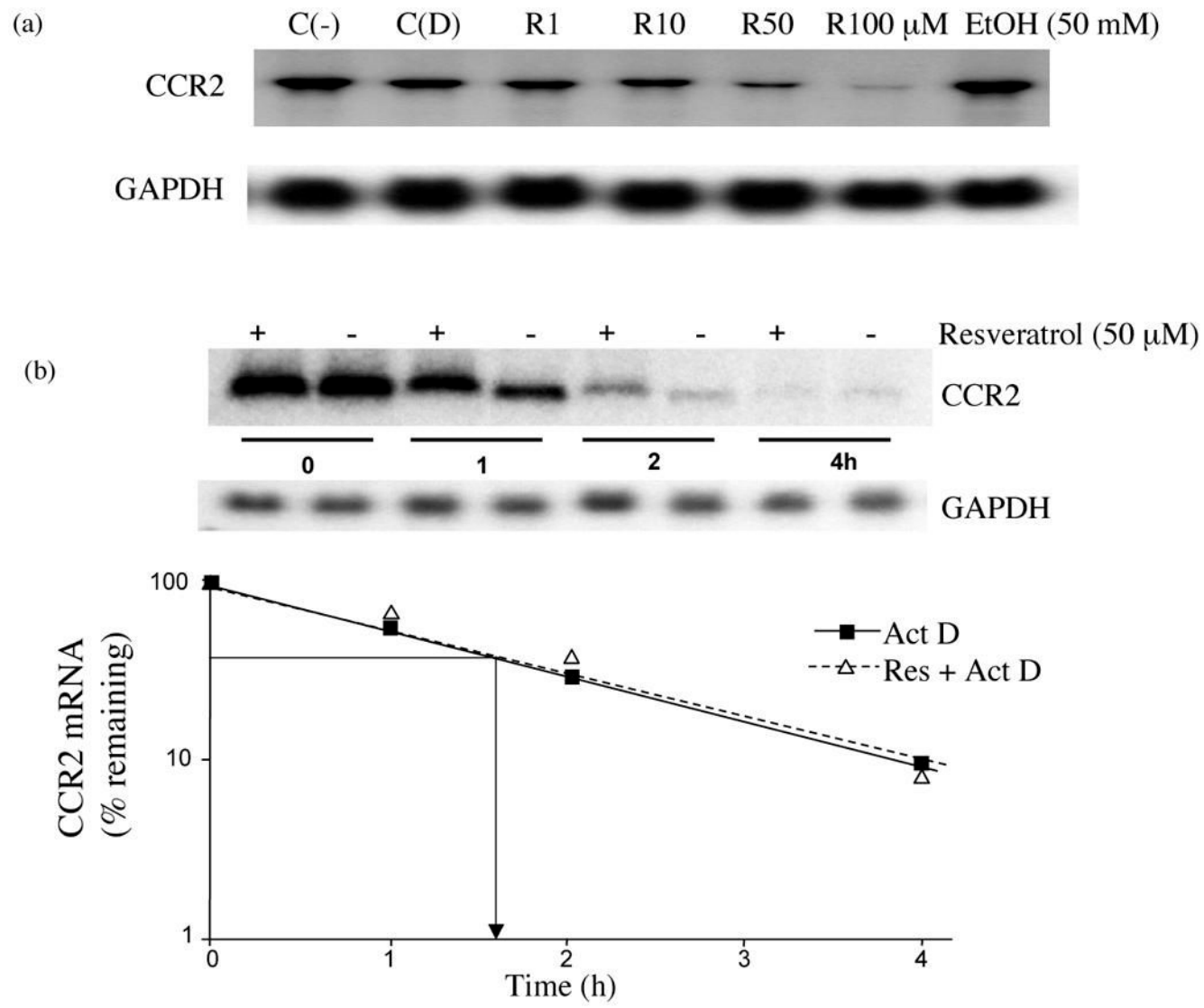

Figure 7.

Resveratrol inhibition of CCR2 mRNA. (a) Representative northern blot showing the effect of resveratrol (R) (1–100μM) on CCR2 mRNA levels in THP-1 cells. The effect of ethanol (EtOH, 50 mM) is shown for comparison purposes. (b) Effect of resveratrol on the stability of CCR2 mRNA expression. THP-1 cells were treated with or without resveratrol (50 μM) and incubated for various periods of time in the presence of actinomycin D (10 μg/ml). Total THP-1 RNA was isolated and northern blots were performed. Top; representative northern blot; bottom, normalized graph (semi-log plot) with values representing means of 3 separate experiments.

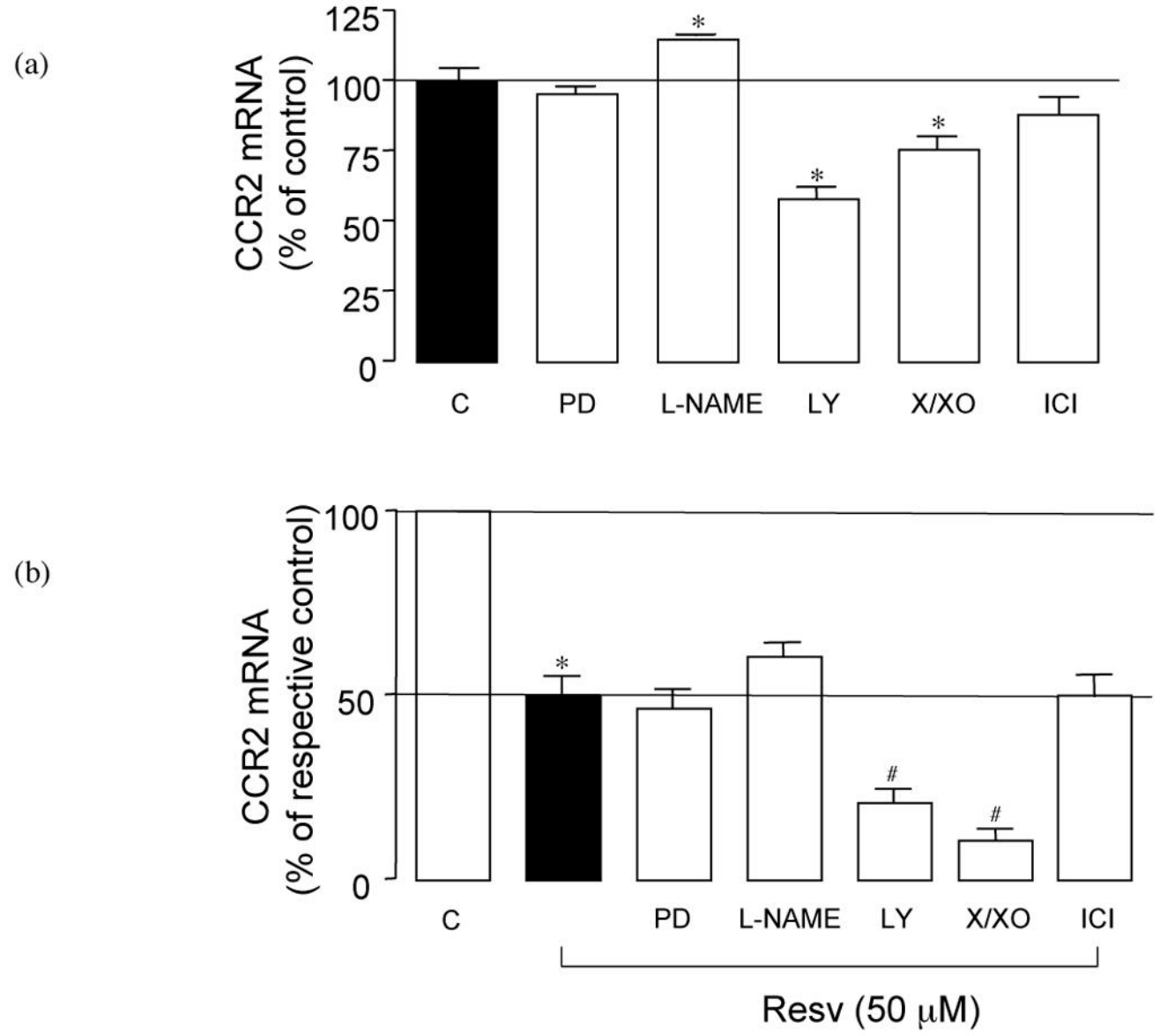

Figure 8.

THP-1 monocytes were preincubated for 30 min with the indicated pharmacological antagonists before treatment with or without resveratrol for 6h. Total RNA was isolated and CCR2 mRNA levels analyzed by Northern blot. (a) The effect of the inhibitors alone, (b) the effect of resveratrol ± the inhibitors. Graphs represents cumulative densitometric data from 3 separate experiments. Mean ± SEM, *$p<0.05$ vs control, #$p<0.05$ vs resveratrol alone.