The dual PPAR-α/γ agonist aleglitazar increases number and function of endothelial progenitor cells, augments neoangiogenesis and vascular function and inhibits atherosclerosis in mice

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Background:

Endothelial progenitor cells (EPC) improve endothelial function and promote vascular repair. We studied the effects of the novel dual peroxisome proliferator-activated receptor (PPAR) - α/γ agonist aleglitazar on EPC, endothelial function, neoangiogenesis and atherosclerosis.

Figure 1
Design of the aleglitazar animal study

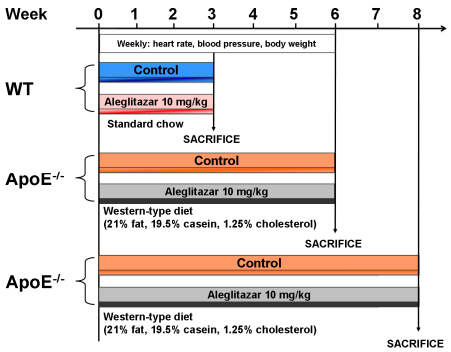
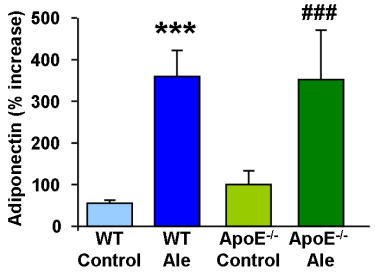
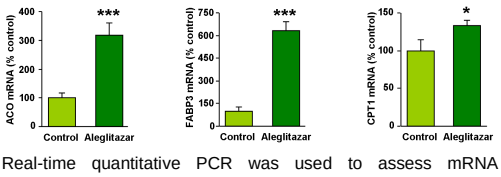


Figure 2
Aleglitazar regulates PPAR-γ downstream targets



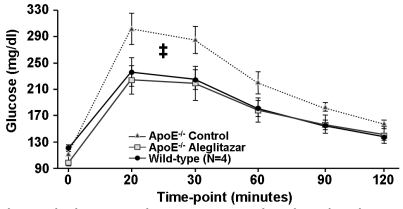
Serum adiponectin concentrations were measured by ELISA before and after treatment in C57Bl/6 WT mice treated with aleglitazar 10 mg/kg i.p. or vehicle daily for 3 weeks and in ApoE$^{-/-}$ mice on Western-type diet treated for 6 weeks (data shown as % change during treatment period). N=6, ***p<0.001 vs. vehicle-injected C57Bl/6 WT mice, ###p<0.001 vs. ApoE$^{-/-}$ controls

Figure 3
Aleglitazar regulates PPAR-α downstream targets



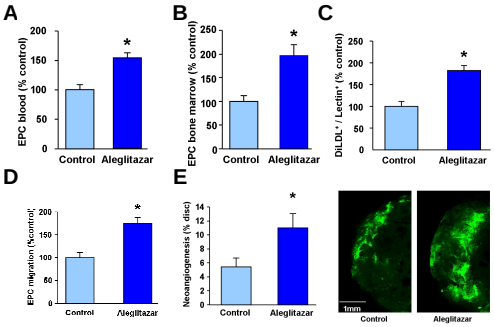
Real-time quantitative PCR was used to assess mRNA expression of PPAR-α target genes ACO, FABP3 and CPT1 in hepatic tissue of ApoE$^{-/-}$ mice on Western-type diet treated for 6 weeks with aleglitazar or vehicle. The 18s mRNA was used as loading control and data were analyzed using the comparative Ct method. N=6, *p<0.05 and ***p<0.001 vs. vehicle-injected ApoE$^{-/-}$ controls.

Figure 4
Aleglitazar normalizes glucose tolerance in ApoE$^{-/-}$ mice on a Western-type diet



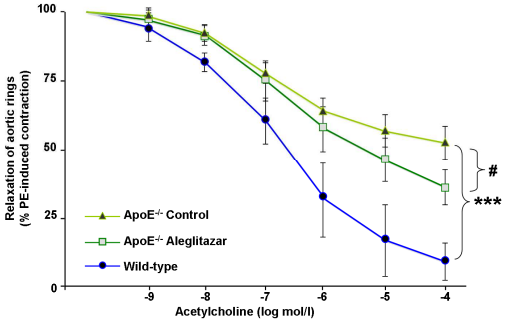
Intraperitoneal glucose tolerance tests showing the time-course of serum glucose after i.p. glucose injection (1.5 mg/kg) in untreated C57Bl/6 WT and ApoE$^{-/-}$ mice on Western-type diet treated with aleglitazar or vehicle daily for 6 weeks. Differences were calculated for the area under the curve (AUC). ‡p<0.001 vs. untreated C57Bl/6 WT mice and vs. ApoE$^{-/-}$ controls.

Figure 5
Number and function of EPC is increased in wild-type mice after 3 weeks aleglitazar treatment



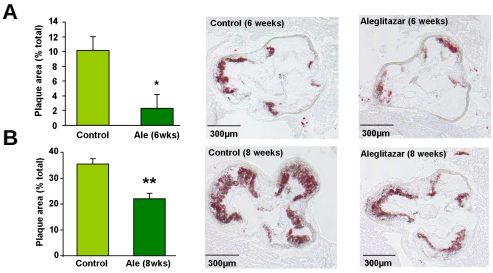
Effects of aleglitazar treatment on the number of Sca-1^{+}/VEGFR-2^{+} EPC in **(A)** the blood and **(B)** the bone marrow as measured by flow cytometry. **(C)** Quantification of spleen-derived diLDL^{+}/lectin^{+} endothelial progenitor cells and **(D)** EPC migration in modified Boyden chambers (100 ng/ml SDF-1 as chemoattractant). **(E)** Quantification of disc neoangiogenesis two weeks after subcutaneous implantation of polyvinyl sponges after perfusion with fluorescent microspheres in mice treated with aleglitazar or vehicle. Right panels show representative images (20 X magnification) of perfused discs. N=6, *p<0.05.

Figure 6
Aleglitazar improves endothelial function in ApoE$^{-/-}$



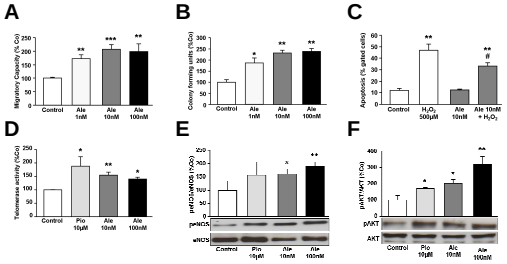
Effects of treatment with aleglitazar daily for 6 weeks in ApoE$^{-/-}$ mice on Western-type diet on the endothelium-dependent vasorelaxation of aortic rings in response to increasing concentrations of carbachol (shown as percentage of maximal phenylephrine-induced constriction) in untreated C57Bl/6 wild-type mice (circles, n=3), ApoE$^{-/-}$ mice after 6 weeks Western-type diet (triangles, n=6), and ApoE$^{-/-}$ mice treated with aleglitazar 10 mg/kg i.p. daily for 6 weeks (rectangles, n=6). ***p<0.001 WT vs. ApoE$^{-/-}$ mice, #p<0.05 for difference between ApoE$^{-/-}$ groups. The were no differences in endothelium-independent vasorelaxation in response to NTG.

Figure 7
Aleglitazar retards development of atherosclerosis



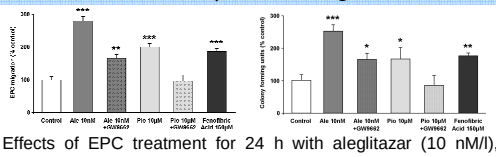
Effects of aleglitazar on atherosclerotic lesions in ApoE$^{-/-}$ mice on a Western-type diet. **(A)** Histomorphometric quantification of atherosclerotic plaques in the aortic sinus after 6 weeks (n=6) or **(B)** 8 weeks (n=7) of aleglitazar, shown as percentage of plaque area / total lumen area. *p<0.05 and **p<0.01 vs. vehicle-treated controls. Images depict Oil red O staining of atherosclerotic plaques (40 X magnification).

Figure 8
Effects of aleglitazar on human endothelial progenitor cells



EPC were isolated from healthy donors by differentiation in cell culture for 4 days and treated as indicated. Effects of aleglitazar treatment for 24 hours on **(A)** EPC migration, **(B)** colony forming units, **(C)** EPC apoptosis (Annexin V FACS comparing basal and hydrogen peroxide-induced apoptosis), **(D)** telomerase activity (TRAP assay), protein expression of **(E)** the ratio of phospho-eNOS/total eNOS and **(F)** phospho-Akt/total Akt. *p<0.05, **p<0.001 and ***p<0.001 vs. controls.

Figure 9
Both, PPAR-α and -γ mediate aleglitazar effects



Effects of EPC treatment for 24 h with aleglitazar (10 nM/l), pioglitazone (10 µmol/L), fenofibric acid (150 µmol/L) and the PPAR- γ antagonist GW9662 (1 µmol/L) on EPC migration in modified Boyden chambers and EPC proliferative capacity as shown by the number of colony forming units. *p<0.05, **p<0.001 and ***p<0.001 vs. controls.

Conclusions:

The dual PPAR-α/γ agonist aleglitazar augments number, function and survival of endothelial progenitor cells, which correlates with improved neoangiogenesis, restored endothelial function and prevention of atherosclerosis. Aleglitazar may benefit patients with cardiovascular diseases beyond its metabolic effects.