REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 18: 175-176 (2004)

HMG-CoA Reductase Inhibitors, but not Calcium Antagonists or Angiotensin Converting Enzyme Inhibitors, Decrease the Uptake of Acetylated-LDL by Human Monocytes

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S u m m a r y. Several lines of evidence indicate that HMG-CoA reductase inhibitors (statins), calcium channel blockers, and angiotensin converting enzyme inhibitors exert antiatherogenic effects in vitro and in vivo. In the present study, we determined the effect of members of the above classes of drugs on the uptake of modified LDL by human monocytes, a key event in the progression of atherosclerosis. U937 cells were treated with drugs and subsequently incubated with fluorescent acetylated-LDL. Fluorescence intensity was assessed by flow cytometry. HMG-CoA reductase inhibitors, but not calcium channel blockers or angiotensin converting enzyme inhibitors, inhibited the uptake of modified LDL by monocytes. Therefore, statins may slow down the progression of atherosclerosis by inhibiting the uptake of modified LDL by monocytes and thus the formation of foam cells.

INTRODUCTION

Atherosclerosis is a vascular disorder that is clinically manifested as coronary heart disease (CHD). The latter is the leading cause of death and disability throughout the developed world. One of the important risk factors for the development of atherosclerosis is dyslipidemia. HMG-CoA reductase inhibitors, widely known as statins, are the drugs of choice for lowering cholesterol. However, they have been shown to possess pleiotropic actions in reducing atherosclerosis (for review see 1). Along with dyslipidemia, hypertension contributes to atherosclerosis in an apparent synergistic manner by common pathways, including endothelial dysfunction. Two classes of antihypertensive drugs, calcium channel blockers (CCBs) and angiotensin converting enzyme (ACE) inhibitors have also been reported to suppress the formation of arterial lesions not only through their antihypertensive action but also by other mechanisms. The antiatherogenic effect of CCBs has been suggested by results of clinical studies (2,3) and by *in vitro* investigations (4-6). Certain cellular changes in the atherosclerotic plaque are characterized by a loss of normal calcium regulation. This observation has led to interest in a potential antiatherosclerotic role of calcium channel blockers, independent of their effects on vasodilation. In addition, similar observations have been made with ACE inhibitors in preclinical (7) as well as clinical studies. The Heart Outcomes Prevention Evaluation (HOPE) study (8) as well as the Study to Evaluate Carotid Ultrasound Changes in Patients Treated with Ramipril and Vitamin E (SECURE) (9) demonstrated that treatment with an ACE inhibitor had beneficial effects on the prognosis and progression of atherosclerosis.

The uptake of modified LDL by monocytes/macrophages is a key event in the progression of atherosclerosis. We sought to determine whether HMG-CoA reductase inhibitors (simvastatin, atorvastatin), CCBs (nifedipine, amlodipine, diltiazem) or ACE inhibitors (enalapril, captopril) reduce the uptake of acetylated-LDL by U937 cells.

METHODS

U937, a monocyte like cell line derived from a patient with histiocytic lymphoma, was purchased from ECACC (U.K.). The cells were cultured in

RPMI-1640 medium (Sigma), supplemented with 10% (w/v) heat-inactivated fetal bovine serum (FBS), 2 mM glutamine (Sigma), and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin) at 37°C in 5% CO_2. In each experiment, $1x10^4$/ml of U937 cells were cultured in RPMI-1640 for 6 days to generate cells in the log phase of growth. The cells were then collected by centrifugation and resuspended in fresh RPMI-1640 at a concentration of $5x10^5$/ml. Subsequently the drugs were added for the indicated incubation time and at various concentrations. An equal volume of drug carrier was added to the control wells. At the end of the incubation the cells were collected by centrifugation and resuspended in serum free RPMI-1640 containing 5 µg / ml Alexa-acetylated LDL (Molecular Probes) for two and a half hours. Untreated cells were also incubated in the absence of Alexa-acetylated LDL to correct for nonspecific staining. Then the cells were washed three times with phosphate buffered saline (PBS) and fixed with 4% formaldehyde (Sigma) in PBS pending flow cytometric analysis.

FACS analysis was carried out with a Becton Dickinson FACS Vantage flow cytometer fitted with an OmniChrome argon laser (Becton Dickinson, Cowley, Oxford, UK). The data were collected in FL-2 and for each analysis the fluorescence intensity of 10000 events was recorded. In order to avoid cell debris, the data were gated on the main population identified on the forward scatter (FSC) and side scatter (SSC) dot plot and analyzed with CellQuest software (Becton Dickinson).

RESULTS

Both simvastatin and atorvastatin inhibited dose-dependently the uptake of acetylated-LDL by U937 cells (Figure1). At 1 µM, both statins showed little inhibition (6-8 %) whereas at 30 µM, the inhibition by simvastatin and atorvastatin was 33% and 38% respectively. The inhibition observed by both statins was statistically significant at 10 µM and 30 µM ($p<0.01$). No cytotoxicity was caused by both statins at the concentrations used as assessed by cell counting.

Incubation of U937 cells with the ACE inhibitors enalapril and captopril (100 µM-1 mM) did not change the uptake of acetylated-LDL by these cells. A lack of effect was also observed after incubation of U937 cells with the CCBs nifedipine (100 µM), diltiazem (100 µM), and amlopipine (0.1 µM-10 µM). The above CCBs caused cytotoxicity at higher concentrations as assessed by cell counting.

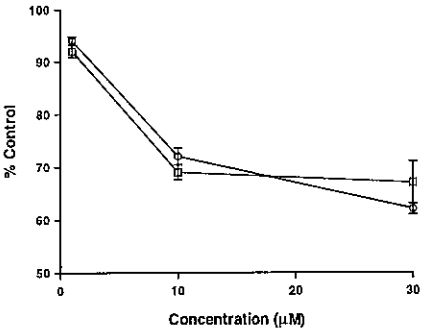

Figure 1 The effect of simvastatin (□) and atorvastatin (○) on the uptake of acetylated-LDL by U937 cells. Results represent mean percentage inhibition (±SEM) of two independent experiments performed in triplicate

CONCLUSIONS

This study demonstrates that HMG-CoA reductase inhibitors, but not ACE inhibitors or CCBs, inhibit the uptake of modified LDL by monocytes. *In vivo*, such cells may represent the monocytes that form foam cells in the intima of blood vessels. This mechanism could contribute to slowing down the progression of atherosclerosis, an effect already ascribed to this class of drugs.

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