HMG-CoA reductase inhibition and PPAR-alpha activation both inhibit cyclosporin A induced endothelin-1 secretion in cultured endothelial cells

Abdelmejid KANDOUSSI†, François MARTIN†, Marc HAZZAN‡, Christian NOËL‡, Jean-Charles FRUCHART†, Bart STAELS† and Patrick DURIEZ††

†Département de Recherches sur les Lipoprotéines et l'Atérosclérose, Inserm U545, Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP 245, 59019, Lille, France, ‡Université de Lille 2, Faculté de Pharmacie, 3 rue du Professeur Laguésse, BP 83, 59006 cedex Lille, France, and ††Clinique Néphrologique, Hôpital Calmette, Rue du Professeur Leclercq, CHRU de Lille, 59037 cedex Lille, France

ABSTRACT

The use of cyclosporin A (CsA) in solid organ transplantation has been shown to be associated with the development of hypertension and nephrotoxicity. Several mechanisms, including endothelin (ET)-1-mediated systemic vasoconstriction, are considered to be responsible for CsA-induced hypertension. This study shows that: (i) incubation of CsA (1 μM) with bovine aortic endothelial cells leads to increased ET secretion by +40%; (ii) the use of compactin, the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor and fibric acid, the peroxisome-proliferator-activated receptor (PPAR)-alpha activator, inhibit the CsA-induced ET secretion to the level below the basal ET secretion, by −32% and −26% respectively; (iii) both inhibitions were reversed by the addition of mevalonate, suggesting communication between the HMG-CoA reductase product and PPAR-alpha pathway. The present findings may be of significant clinical relevance, since statins and fibrates beyond their hypolipidaemic action may represent a potential therapeutic tool in the treatment or prophylaxis of CsA-associated side effects. Furthermore, we suggest that the mevalonate metabolism would interfere with PPAR-alpha activity.

INTRODUCTION

Cyclosporin A (CsA), a fungal cyclic polypeptide, is an immunosuppressive drug that acts primarily on T-lymphocytes. The clinical use of CsA has provided new perspectives in organ transplantation. However, the extensive use of CsA has been accompanied by potentially severe side effects such as hypertension and renal hypoperfusion. Previous investigations have indicated that endothelin-1 (ET-1), a vasoactive peptide, may be a mediator of CsA-induced renal vasoconstriction and nephrotoxicity [1].

Statins, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, have been shown to be efficacious in reducing cardiovascular morbidity and mortality in primary [2] and secondary prevention [3] clinical trials. The beneficial effects of HMG-CoA reductase inhibitors are not necessarily related to a detectable decrease in serum cholesterol levels, and it is now demonstrated that the pleiotropic effects of HMG-CoA reductase inhibitors and PPAR-alpha activation...
CoA reductase inhibitors participate in the capacity of these molecules to reduce the coronary heart disease risk.

Fibrates, peroxisome-proliferator-activated receptor (PPAR)-alpha activators, form the second major class of hypolipidaemic drugs, which are very active in reducing high triacylglycerol and in increasing low HDL-cholesterol plasma levels, a common feature in chronic renal failure [4] and in kidney transplant patients [5]. Over the last 3 years it has been clearly demonstrated that PPAR-activators share most of the pleiotropic effects of HMG-CoA inhibitors: inhibition of vascular wall inflammation, anti-thrombotic effects (inhibition of tissue factor secretion), inhibition of ET-1 secretion [6], etc.

Using bovine aortic endothelial cells (BAEC), we have investigated if these drugs could modulate the ET-1 secretion induced by CsA and the mechanism by which they act.

**METHODS**

**Cells**

BAEC were obtained by scraping the aorta excised from a freshly slaughtered cow. The cells were maintained and cloned. They were cultured in Dulbecco’s modified Eagle’s medium (DMEM), containing basic fibroblast growth factor (1 ng/ml) with new born calf serum (10% v/v). Cells were grown in 35-mm-diameter dishes. The cultures used in this study had undergone six passages. The cultured endothelial cells had a typical morphology and homogeneous staining for factor-VIII antigen.

**Experimental protocol**

Confluent cells were preincubated in triplicate for 6 h in a total volume of 1 ml of DMEM with 10% (v/v) lipoprotein deficient serum with ciprofibrate (50 µM) or compactin (5 µM) (Sigma, MO, U.S.A.) or vehicle. Following this period, cells were incubated with CsA in the presence or in absence of mevalonate 100 µM (Sigma) for 16 h.

**Measurement of ET**

At the end of the treatment period, an Endothelin-1,2 (high sensitivity) 125I-radiosystem* (Amersham, les Ullis, France) was used to quantify immunoreactive ET in the culture medium. Results were normalized to cellular protein content in all experiments and expressed as a percentage relative to the cells incubated with the vehicle.

**RESULTS**

CsA (1 µM) increased the basal (BAEC in presence of vehicle alone) ET-1 secretion by +40% over the stimulation period of 16 h. Incubation of the cells with 5 µM compactin alone reduced basal ET-1 secretion by -21%. Incubation of the cells with both CsA and compactin led to more pronounced decrease in ET-secretion of -32%. Fibric acid (50 µM) did not significantly reduce the basal secretion of ET-1. However in the presence of CsA, fibric acid reduced ET-1 secretion by -26% (Figure 1).

The role of the HMG-CoA pathway on compactin and fibric acid mediated inhibition of CsA induced ET-1 secretion was examined by the addition of 100 µM mevalonate. Mevalonate partially reversed the inhibitory effect of compactin and fibric acid on CsA induced ET-1 secretion from -32% and -26% to +24% and +7% respectively (Figure 2).
**DISCUSSION**

CsA binds to cyclophilin and inhibits calcineurin catalytic activity which is a Ca$^{2+}$-dependent serine/threonine phosphatase. In the absence of CsA, one substrate of activated calcineurin, a cytosolic component of NFAT (NFATc; nuclear factor of activated T-cells) moves from the cytoplasm to the nucleus upon dephosphorylation [7]. By associating with another nuclear component of NFAT (NFATn), this calcineurin substrate regulates the expression of many genes. In the presence of CsA, the resulting inhibition of calcineurin impedes the translocation of NFATc into the nucleus, thus inhibiting the induction of numerous genes [7].

CsA increased reactive oxygen species accumulation in BAEC, and the participation of a redox-sensitive potential is involved in activator protein-1 (AP-1) activation [8]. ET-1 promoter activity has been shown to be controlled by a complex interaction of GATA and AP-1 transcription factors binding to closely opposed sites on the proximal ET-1 promoter [9]. Thus it seemed reasonable to suggest that the reactive oxygen species generated by CsA could serve as an intermediate transducer of the effect of CsA in BAEC.

NFAT is also noted for its ability to bind cooperatively with transcription factors of the AP-1 (Fos/Jun) family to form the composite NFAT/AP-1 [10]. In this study, a PPAR-alpha activator (ciprofibric acid) slightly inhibits basal ET-1 secretion by −4%. We have already reported that PPAR-alpha activators (fenofibric acid and Wy-14643) tend to decrease basal ET-1 secretion [11] and that activated PPAR-alpha interferes negatively with the AP-1 signalling pathway to inhibit thrombin activation of ET-1 gene transcription [11]. Therefore, we suggest that in basal conditions (absence of CsA) but in the presence of a PPAR-alpha activator (ciprofibric acid), constitutive ET-1 gene transcription is repressed through the combined inhibitory effects of both activated PPAR-alpha (this is a demonstrated assertion) and of NFAT (this is a putative assertion) on AP-1 induced ET-1 gene transcription.

In the presence of CsA, a PPAR-activator (ciprofibric acid) dramatically reduced ET-1 secretion, below levels measured in basal conditions (−26%). We suggest that NFAT is a poor inhibitor of AP-1-induced ET-1 gene transcription, while activated PPAR-alpha is shown to be a strong inhibitor [11]. Therefore, by decreasing NFAT concentration in the nucleus, CsA would allow activated PPAR-alpha (a strong inhibitor of AP-1 induced ET-1 gene transcription [11]) to dramatically substitute for NFAT (a poor putative inhibitor of AP-1 induced ET-1 gene transcription) and to greatly inhibit free AP-1 induced ET-1 gene transcription. This in turn would result in the dramatic decrease in ET-1 secretion that we have observed.

The inhibitory effects of compactin and fibrates on CsA induced ET-1 secretion were reversed by the exposure of BAEC to mevalonate, the product of enzymatic conversion of HMG-CoA, suggesting the existence of a new regulatory level at which statins and fibrates may modify cellular homeostasis of vasoactive mediators. The mechanisms whereby these effects take place remain to be elucidated. Further studies using isoprenoids, the different metabolite compounds of cholesterol biosynthesis are needed.

The findings of this study may be of significant clinical relevance, since statins and fibrates beyond their hypo- lipidaemic action, may represent a potential therapeutic tool in the treatment or prophylaxis of CsA-associated side effects.

**ACKNOWLEDGMENTS**

This work was supported by Santelys Association, Chemin de Tournai, Épi De Soil, Lille.

**REFERENCES**