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Letter to the Editor

Reduction of endoplasmic reticulum stress—A novel mechanism of action of statins in the protection against atherosclerosis

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ABSTRACT

Fatty-acid-induced endoplasmic reticulum stress has been recently described as a novel mechanism involved in the genesis of atherosclerosis. Here we show that statins, a class of drug widely employed in the clinical management of hypercholesterolemia, reduces lipid-induced macrophage endoplasmic reticulum stress in an isolated cell system and in LDL receptor knockout mice. Given the importance of endoplasmic reticulum stress as an inducer of inflammation, we suspect that the novel mechanism of action herein described for statins may play a major role on its beneficial effects in the prevention of cardiovascular disease.

Dear Editor,

In a recently published study [1], strong evidence was presented to support a role for endoplasmic reticulum stress (ER-stress) on the genesis of atherosclerosis. Accordingly, the chaperone aP2 mediates lipid-induced ER-stress in macrophages and the inhibition of this protein by chemical or genetic approaches mitigates ER-stress and alleviates atherosclerosis.

In clinical practice, the HMG-CoA reductase inhibitors, statins, are widely used to control atherosclerosis providing up to 60% reduction in the number of cardiac events [2] and almost 20% reduction in the risk of stroke [3]. The inhibition of the first step of the cholesterol synthetic pathway is regarded as the main mechanism of action of the statins [4]. However, as the clinical use of statins leads to only 25–30% reduction of baseline LDL-cholesterol, it was predicted that other effects should contribute to the outstanding clinical outcomes provided by this class of drug. Indeed, a number of studies showed that besides its primary role on cholesterol lowering, statins reduced inflammation, increased plaque stability and improved endothelial function [5]. Nevertheless, the molecular mechanisms behind all these pleotropic effects are not completely elucidated. Here we show that statins prevent fatty-acid-induced endoplasmic reticulum stress in a macrophage cell line and reduce ER-stress in activated macrophages present in the arterial walls of an animal model of atherosclerosis.

The treatment of the monocyte/macrophage cell line Thp-1 with long-chain saturated fatty acids, such as stearate (SA, Fig. 1) or palmitate (not shown), induce ER-stress as determined by the increased phosphorylation of the ER membrane kinase PERK (PKR-like endoplasmic reticulum kinase) (Fig. 1A, upper blot; and Fig. 1B) and its substrate, eIF2α (eukaryotic translation initiation factor 2α) (Fig. 1A, blot in the middle; and Fig. 1C), the increased expression of the spliced form of the transcription factor XBP-1 (X-box binding protein-1) (Fig. 1A, lower blot; and Fig. 1D) and also of its precursor mRNA (Fig. 1E), and the increased expression of the chaperone GRP78 (78 kDa glucose-regulated protein) (Fig. 1F and G). The treatment of the cells with either simvastatin (Sim) or pravastatin (Pr), significantly reduced the expression of all markers of ER-stress, independently of the pathway analyzed (Fig. 1A–G). The effect of the statins to inhibit ER-stress was apparently dependent on its control of HMG-CoA reductase activity because bypassing the inhibition of the enzyme with mevalonate hampered the inhibitory effect of pravastatin upon ER-stress (Fig. 1H–L). In addition, the treatment of an animal model of atherosclerosis, the LDL receptor deficient mouse, LDLR (−/−) [6], with pravastatin, significantly reduced the coexpression of P-eIF2α with F4/80 in the arterial wall indicating a reduction of ER-stress in activated macrophages in a site of atherosclerotic plaque formation (Fig. 1M–O).

At least one previous study has shown that statins can induce some degree of activation of ER-stress in macrophages [7]. Indeed, in our hands, the treatment of naive Thp-1 cells with either simvastatin or pravastatin promoted a discrete, but significant increased in the activation of all markers of ER-stress analyzed (P-PERK, P-eIF2α, XBP-1 and GRP78, not shown). However, once Thp-1 cells were chased with fatty acids, the level of expression of ER-stress markers increased outstandingly, an effect that was abrogated by the statins.

Interestingly, a recent study [8] showed that when ER-stress is induced in the heart by pressure overload, the beneficial effect of pravastatin on cardiac remodeling is accompanied by reduction of ER-stress, suggesting that statins can inhibit ER-stress in different clinical contexts and not only when induced by fatty acids.

Thus, inhibition of ER-stress in macrophages is yet another mechanism of action of this highly pleotropic class of drug. As mevalonate overcame the effect of statins on ER-stress, it seems that, at least part of the anti-ER-stress action of statins depends on its classical mechanism of action.

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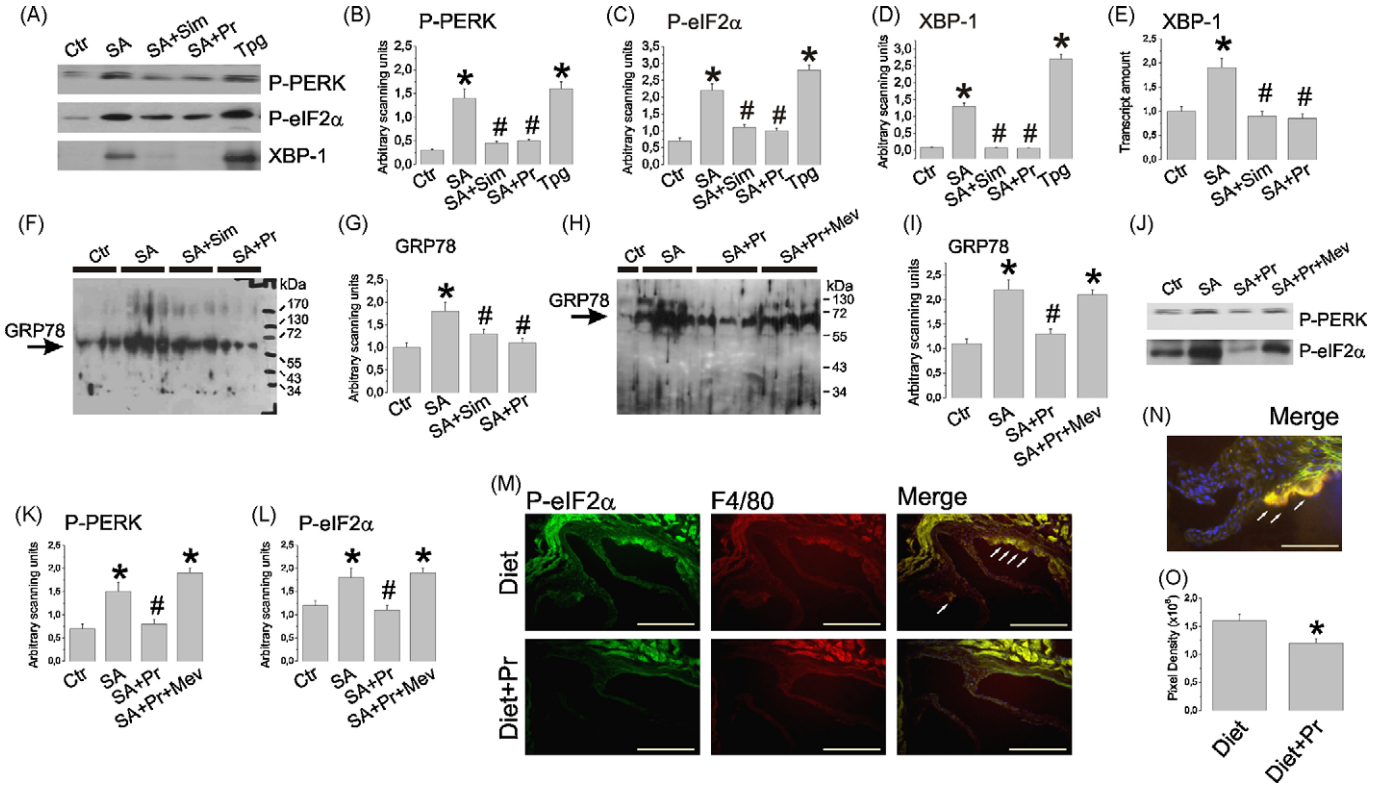

Fig. 1. (A)–(L) Thp-1 cells were plated to confluence (10^7 cells/plate) in RPMI and treated for 24 h either with vehicle (Ctr, ethanol to a final concentration of 0.05%), or stearic acid (SA, 100 μM), or SA + simvastatin (SA + Sim, 100 μM + 2 μM, respectively), or SA + pravastatin (SA + Pr, 100 μM + 2 μM, respectively) or tapsigargin (Tpg, 2 μM) or SA + Pr + mevalonate (SA + Pr + Mev, 100 μM + 2 μM + 100 μM, respectively); phosphorylated forms of PERK (P-PERK) and eIF2α (P-eIF2α), and spliced form of XBP-1 were determined by immunoblot of 40 μg protein extracts separated by SDS-PAGE (A) and (J); quantification of specific protein bands are depicted in (B)–(D) for P-PERK, P-eIF2α and XBP-1, respectively, and (K) and (L) for P-PERK and P-eIF2α, respectively. The transcript amount of the spliced form of XBP-1 was determined by PCR (E). Expression of GRP78 was determined by immunoblot of 40 μg protein extracts separated by SDS-PAGE (F) and (H) and quantifications of specific bands are depicted in (G) and (I). (M)–(O) LDL receptor knockout mice were treated for 10 days with a diet containing 40% fat (predominantly saturated fat from lard) (Diet) or with the same diet plus pravastatin (15 mg/kg day, Diet + Pr) for 10 days; at the end of the experimental period the aorta was obtained for immunfluorescence staining with anti-P-eIF2α (green) and anti-F4/80 (red). Co-immunolocalizations (arrows) of P-eIF2α and F4/80 are depicted in M, Merge, and in N, at higher magnification. Quantification of areas presenting double staining was performed by the Image-J software (http://rsbweb.nih.gov/ij) and results are depicted in O. In all experiments $n = 6$. In M, bars = 100 μm; in N, bars = 25 μm. In (B)–(E), (J), (I) and (K), (L), $^*p < 0.05$ vs. Ctr; $\#p < 0.05$ vs. SA. In (O) $^*p < 0.05$ vs. Diet. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

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