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SHORT COMMUNICATION

Direct effects of rosuvastatin on pancreatic human beta cells

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Introduction

The 3-hydroxy-methylglutaryl coenzyme A inhibitors statins are largely used for primary and secondary prevention of atherosclerotic cardiovascular disease in both non-diabetic and diabetic patients [1, 2]. However, recent work has suggested that statin therapy may be associated with increased risk of new onset diabetes and deterioration of glycemic control. The concern was initially raised in 2008, when increased incidence of diabetes among patients taking rosuvastatin in the JUPITER study was reported [3]. A successive meta-analysis of randomized placebo-controlled and standard care–controlled trials (19,140 subjects of whom 4,278 developed diabetes) demonstrated a 9 % increased risk of incident diabetes in statin-treated individuals [4]. The benefits of statin treatment largely exceed the diabetes hazard [5–7]; nevertheless, it is important to investigate the mechanisms through which these molecules might affect glucose homeostasis. In this study, we have assessed the direct action of rosuvastatin on isolated human islets. Insulin secretion, beta cell survival, ultrastructure and gene expression studies were performed.

Materials and methods

Islet isolation and culture

Islets were prepared from 7 multi-organ non-diabetic donors (age: 65 ± 15 years; gender: 5M/2F; BMI: 27.9 ± 4.8 kg/m^2) by collagenase digestion followed by density gradient purification [8–10]. After isolation, the islets were maintained for 2–3 days in M199 medium, containing 5.5 mmol/l glucose, supplemented with 10 % serum and antibiotics. Then, batches of approximately 1,000 islets were cultured for 24 h in M199 medium either with or without the addition of 1, 10 or 100 ng/ml rosuvastatin (encompassing therapeutical concentrations). In additional experiments, islets were incubated for 24 h with 0.5 mmol/l palmitate, in presence or not of 10 ng/ml rosuvastatin.

Functional studies

For insulin secretion studies [8], islets were first kept at 37 °C for 45 min in Krebs–Ringer bicarbonate solution (KRB), 0.5 % (vol./vol.) albumin, pH 7.4, containing 3.3 mmol/l glucose (wash-out phase). Then, the medium was replaced with KRB containing 3.3 mmol/l glucose to assess basal insulin secretion (45 min), followed by a further 45 min incubation with 16.7 mmol/l glucose to assess insulin response to acute challenge. Insulin was quantified using an immunoradiometric assay (Pantec, Turin, Italy).

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Electron microscopy

Electron microscopy experiments were performed as previously described [9, 11]. Tissue was fixed with 2.5 % glutaraldehyde in 0.1 mol/l phosphate buffer, pH 7.4, for 2 h at 4 °C. After rinsing in phosphate buffer, samples were postfixed in 1 % phosphate-buffered osmium tetroxide for 30 min at 4 °C, then dehydrated in a graded series of ethanol, transferred to propylene oxide and embedded in Poly/bed 812 (Poliscience, CA, USA). Ultrathin sections (60–80 nm thick) were cut with a diamond knife, placed on copper grids (200 mesh), and stained with uranyl acetate and lead citrate. Micrographs were obtained at 10,000 magnifications. The volume density (milliliter percent) of the granules was calculated according to the formula: $VD = p/Pt$, where p is the number of points within the subcellular component and Pt is the total number of points.

Gene expression studies

To perform gene expression experiments [8], total RNA was extracted using the RNeasy mini Kit (Qiagen, Milano, Italy) according to manufacturer's recommendations and quantified by absorbance at A260/A280 nm (ratio >1.9) in a NanoDrop 2000C. RNA integrity was assessed with an Agilent 2100 Bioanalyzer. For quantitative PCR experiments, total RNA was reverse transcripted with iScript cDNA Synthesis Kit. The oligonucleotides of interest for superoxide dismutase 2 and catalase were obtained from assay-on-demand gene expression products (Life Technologies, Carlsbad, CA, USA). The mRNA level of target genes was quantified and normalized for β-actin in an Applied Biosystem 7700 bioanalyzer.

Statistical analysis

Results are given as mean ± SD. Differences between groups were assessed by the two-tailed paired or unpaired Student's t test, or the ANOVA test with Bonferroni correction, as appropriate.

Results

In non-treated, control islets, insulin secretion induced by 3.3 and 16.7 mmol/l glucose was 46.4 ± 26.6 μU/ml and 92.4 ± 21.9 μU/ml ($p < 0.01$ vs. 3.3 mmol/l glucose), respectively, with a stimulation index (stimulated over basal insulin release) of 2.4 ± 0.8. Rosuvastatin alone did not cause any significant change in basal and stimulated insulin release, so that stimulation index values were 2.8 ± 0.2 with 1.0 ng/ml, 2.6 ± 1.7 with 10 ng/ml and 2.4 ± 0.7 with 100 ng/ml. Incubation for 24 h with 0.5 mmol/l palmitate caused a significant decrease of glucose-stimulated insulin secretion, with a stimulation index of 1.7 ± 0.8 ($p < 0.05$ vs. control islets by the paired t test). However, the presence of 10 ng/ml rosuvastatin in the 0.5 mmol/l palmitate medium prevented the fatty acid–induced derangement of insulin release (stimulation index: 2.1 ± 0.4, NS vs. control islets). In addition, by electron microscopy, it was found that the percentage of apoptotic beta cells [9, 11] was higher ($p < 0.05$ by the Bonferroni correction) in palmitate-exposed islets (2.0 ± 0.9 %, number of cells counted: 153) than in control islets (1.0 ± 1.0 %, number of cells counted: 212), which was prevented by the presence of the statin in the palmitate-containing medium (0.3 ± 0.3 %, number of cells counted: 148). Ultrastructure analysis results are illustrated in

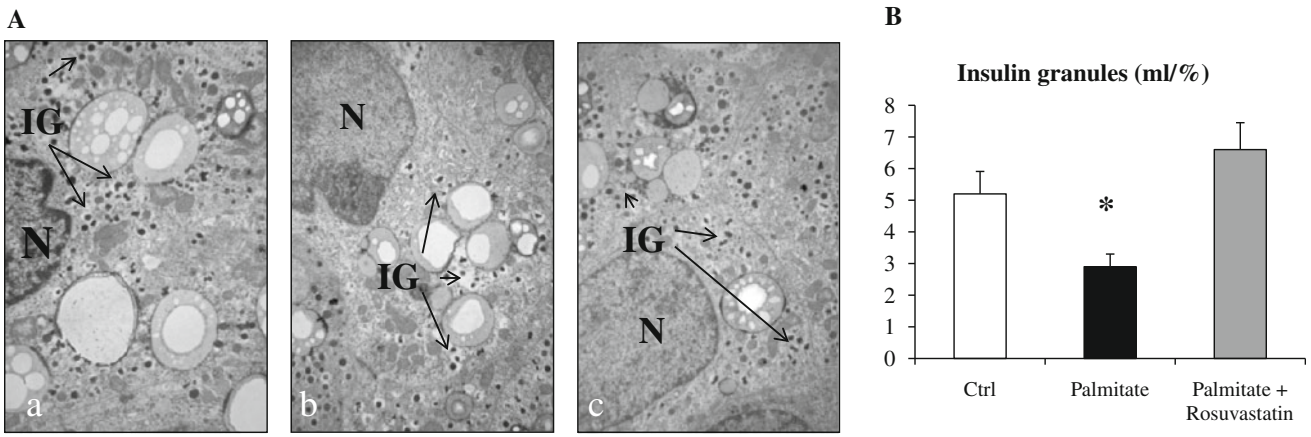

Fig. 1 **A** micrographs of beta cell from control islets (*a*) and islets exposed to 0.5 mmol/l palmitate either without (*b*) or with rosuvastatin (*c*). *N* nucleus; *IG* insulin granules. Magnification: × 10,000. **B**: quantification of insulin granule volume density in beta cells from islets incubated for 24 h under the different conditions reported for **A**. *Ctrl* controls. *$p < 0.05$ versus other groups by the Bonferroni correction

Fig. 1: compared to control cells, palmitate-exposed beta cells showed marked insulin degranulation, which was counteracted by rosuvastatin. Gene expression of superoxide dismutase 2 and catalase was measured by qPCR with islets under the different experimental conditions, and it was only marginally affected by palmitate and rosuvastatin (not shown).

Conclusions/interpretation

Recent reports indicate that statins are associated with increased risk of diabetes, an effect that seems to be dose dependent [12]. Whether the various statins may have different roles in this regard is still matter of debate [4, 12]. In the present study, we report that rosuvastatin had no direct deleterious effect on islet insulin secretion from human islets at doses from 1 to 100 ng/ml. Actually, the molecule seemed able to protect beta cells from lipotoxicity, as suggested by improved insulin secretion, beta cell survival and beta cell ultrastructure in the presence of the statin. From our study, it is not possible to infer on the mechanisms involved in rosuvastatin-protective action against palmitate toxicity, which, however, did not seem to be due to changes in the expression of genes involved in redox balance. Our experiments were accomplished after 24-h incubation with the statin. Clearly, more prolonged beta cell exposure to rosuvastin and other molecules of the same family are warranted to definitely assess the direct action of statins on the human beta cell.

Conflict of interest None.

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