7βhydroxycholestrol is Incorporated in Caveolae from Endothelial Cells.

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Introduction: Caveolae are invaginations in endothelial cell membrane, where a large number of signaling proteins are located, including CD40, eNOS. Caveolae are characterized by their high cholesterol content and the presence of caveolin-1 (CAV-1), a scaffolding protein for molecules belonging to signaling pathways. Oxysterols are derived from cholesterol oxidation; they may modify gene transcription by binding to the nuclear receptor LXR and are known to be present in atherosclerotic plaque. We hypothesized that oxysterols may act also by changing caveolae structure and, therefore, changing signal transduction and cell function. Methods: We exposed human umbilical vein endothelial cells (HUVECs) to 7βhydroxycholesterol (10μM/mL) for 1h. Membrane sub-fractions containing caveolae were obtained by ultracentrifugation in a sucrose gradient and its composition was analysed by mass spectroscopy. In culture medium, TNFα and IL-10 were assessed by ELISA. CD40 and CAV-1 mRNA was analyzed by RT-PCR. Results: The chromatographic profile of HUVECs' fractions rich in caveolae showed a much higher content of oxystherols in treated cells, compared to controls. There was no difference in mRNA for either CD40 or CAV-1 compared to control. Exposure to oxysterols did not affect either TNFα or IL-10 secretion compared to control (79.7±2.3 vs. 94.2±0.3 pg/mL and 35.5±2.2 vs 49.6±16.9 pg/mL, respectively). Conclusion: 7βhydroxycholesterol is incorporated in endothelial cells caveolae after 1h treatment. Under these experimental conditions, 7βhydroxycholesterol did not affect the expression of genes involved in the inflammatory response, therefore, any effects on the activity of these signaling pathways observed after oxysterol exposure might be related to its impact in caveolae structure. Financial support: CAPES, FAPESP.