

Glucocorticoid new pro-atherogenic activity mediated by cell cholesterol accumulation: study on molecular mechanisms and potential counteracting strategies.

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Glucocorticoids (GC) represent the main prescribed therapy for the treatment of inflammatory disorders and autoimmune diseases, such as rheumatoid arthritis and systemic lupus erythematosus, but long-term steroid treatment is associated with increased prevalence of cardiovascular events. Although this association has been attributed so far to the well-described systemic metabolic effects of GC, a recent study showed that chronic steroid therapy is an independent cardiovascular risk factor, not related to dyslipidaemia or hypertension.

The aim of the study is to investigate whether GC exert a direct effect on macrophage cholesterol metabolism, leading to the promotion of foam cell formation, the main cell type involved in atheroma development.

THP-1 derived macrophages, normal and low density lipoprotein receptor (LDLr)-lacking fibroblasts, and human smooth muscle cells (SMCs) were incubated with hydrocortisone (HC) at various concentrations. HC was chosen for its hydrosolubility and *in vitro* direct activity. Cell cholesterol content and cholesterol efflux were measured through fluorimetric and radioisotopic techniques respectively; LDL uptake was directly observed by confocal microscopy technique; mRNA expression of *ldlr* and *cd36* gene was evaluated by real-time PCR; the protein expression of LDLr, of cholesterol transporters ATP binding cassette A1 (ABCA1) and G1 (ABCG1), of Scavenger Receptor Type B Class I (SR-BI), of CD36 and of LXR/RXR receptor was evaluated by western blotting. Macrophage cholesterol esterification was measured with radioisotopic technique and thin layer chromatography.

We observed that HC promotes a dose-dependent cholesterol accumulation in macrophages ($r^2 = 0.848$, $p < 0.01$). Then we were able to demonstrate three mechanisms contributing to such effect. The first is the increase of LDL internalization velocity and degree in macrophages, effect mainly mediated by augmented mRNA transcription and protein expression of LDLr. Second, we observed that HC reduces cholesterol efflux in a dose dependent manner ($r^2 = 0.688$, $p < 0.001$), both in cells in basal conditions and in cells with pharmacologically activated LXR, by reducing activity and expression of ABCA1, ABCG1 and SR-BI cholesterol transporters for cell cholesterol efflux and their regulator LXR/RXR nuclear receptor.

Third, we observed that HC increases cholesterol esterification in cholesterol loaded macrophages (226% increase, $p < 0.0005$), promoting cholesterol storage and limiting its availability for efflux.

Similar effects were demonstrated on SMCs, on which HC significantly reduced cholesterol efflux (mean \pm SEM: 2.8 ± 0.18 and $2.1 \pm 0.07\%$ HC-untreated cells versus HC-treated cells $p < 0.05$) and increased cholesterol influx (μg cholesterol /mg protein mean \pm SEM: 17.65 ± 1.8 and 44.9 ± 0.76 HC-untreated cells versus HC-treated cells, $p < 0.001$).

Our study showed that HC exerts pro-atherogenic effect by directly promoting cholesterol accumulation in macrophages through various molecular pathways and inducing the differentiation of SMCs to a macrophages-like phenotype. In fact, these effects might be particularly relevant on vessel cells, in constant contact with circulating lipoproteins. These observations provide important information not only to understand the mechanisms of steroid-associated cardiovascular risk, but also to possibly design drugs with a lower impact on macrophage cholesterol metabolism or to develop combination treatment with molecules able to counteract cholesterol macrophage accumulation.