Role of catestatin as such as slowly released by fibronectin-coated pharmacologically active microcarriers (Fn-Pam) in limiting hypoxicinduced cell death

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Résumé en anglais	Objectives: Catestatin (CST), a 21-amino acid derivate of Chromogranin A, exerts several biological functions, including inhibition of catecholamine release and cardioprotective role. Moreover positive effect of CST on monocyte migration in vitro and the induction of angiogenesis, arteriogenesis and vasculogenesis in the mouse hind limb ischemia model have been demonstrated. Collateral arteries may provide a biological bypass for occluded atherosclerotic vessels, increasing blood flow to ischemic tissue. In such a prospective, CST is a very promising agent for revascularization purposes, in “NO-OPTION” patients. However, proteins have a very short half-life after administration and must be conveniently protected. FN-PAMs, biodegradable and biocompatible polymeric microspheres, have ideal characteristic for this purpose: besides to convey peptides and allow in situ prolonged/controlled delivery, they may also convey cells on their biomimetic surface and may favor their survival and engraftment after cell transplantation. In this study, we show that CST can be incorporated within FN-PAM and aim to demonstrate that CST may be released in a slowly/prolonged manner by FN-PAM. We also aim to demonstrate that CST released by FN-PAM may reduce cell death under different stress conditions. Materials and methods: CST has to be precipitated to ensure its stability upon subsequent encapsulation. Protein precipitate is formed from aqueous solution by the addition of a watermiscible organic solvent. PLGA–P188–PLGA (triblock) copolymeric microspheres are prepared using solid/oil/water emulsion solvent evaporation–extraction technique. PAMs are coated with Fibronectin and characterized by Immunofluroscence (confocal microscopy). Mesenchymal stem cells (MSC) are exposed to hypoxia (72 h in 1–2%O2) and reoxygenation (6 h in 21% O2) in a hypoxic chamber with or without CTS, FN-PAMs or CTS-FN-PAMs. The protective effects of treatments are detected by MTT assay. Results: To define the optimum condition of nanoprecipitation we used an experimental design, modifying parameters influencing protein precipitation: ionic strength, mixing and centrifugation time. Nanoprecipitation of CST was found to be 72%. Controlled release of CST from CTS-FNPAM greatly limits hypoxic MSC death and enhances MSC survival in post-hypoxic environment. Conclusions: FN-PAMs are successfully formulated with CST. By an experimental design, we found optimal conditions to obtain a good CTS nanoprecipitation yield. MSC readily adhere to the FN-PAM and CST-FN-PAMs reduce MSC death enhancing survival in post-hypoxic environment. Data suggest that CST-FN-PAMs are promising tools for therapeutic purpose.
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