# A pharmacological role of curcumin in the protection from oxidative stressof mesenchymal stem cells of mouse origin

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Mesenchymal stem cells (MSCs) are a promising tool to improve tissue repair. The administration of these cells has shown able to contribute to the regeneration of different tissues in organ as, for example, heart, kidney and lung (Monsel et al., 2014). Although these findings are encouraging, low survival and partial engraftment and migration are still practical problems for an extensive in vivo use (Haque et al., 2015). Damaged tissue is a problematic site of engraftment since is often characterized by a necrotic and oxidative microenvironment. Some authors have reported that conditioned stem cells obtained by inducing cytoprotective pathways may improve their therapeutic potential (Yagi et al., 2013). We have characterized a spontaneously immortalized cell line, named m17.ASC, that was derived from adipose tissue of adult FVB/N mice (Zamperone et al., 2013). This stable and not tumoral cell line could be adopted to investigate possible pharmacological modulations and give the possibility to conduct effective conditioning for cytoprotection. In the last years antioxidants from natural origin have received an increasing attention. Curcumin, from curcuma longa (turmeric), is a natural antioxidant molecule with a wide range of potential pharmacological activities in many diseases (Kapakos et al., 2012). In this study, we analyzed if curcumin could exert beneficial activities on MSCs when exposed to oxidative stress. We used two different schemes of treatment, where cells were treated with curcumin before (pre-conditioning) and after the oxidative insult. m17.ASC were treated with different concentrations of curcumin ranging from 0.5 to 5 μM, and challenged with increasing concentrations of $H_2O_2$ (0–1000 μM) to mimic oxidative stress conditions. We analyzed how preconditioning or post stress treatment with curcumin affected overall viability of the cells or regulated the expression of genes and proteins involved in the apoptotic pathways. $H_2O_2$ caused a dose-dependent reduction of cell viability (MTT assay), and an increase of the number of apoptotic cells (Annexin V/Propidium iodide assay), as expected. Curcumin exerted an effective protective effect against stress insult, since it preserved the proliferative potential and reduced the number of apoptotic m17.ASC cells, in a dose-dependent manner, only when these were pre-conditioned (24 hours of continuous exposure). If curcumin was administered after $H_2O_2$ challenges, no significant protection was showed at any concentration. The treatment with hydrogen peroxide was able to induce the activation of proteins Caspase-3 and Caspase-9 in a dose-dependent manner, as demonstrated by Western-Blot analyses. Moreover, curcumin pre-conditioning was able to prevent caspases activation. We performed qRT-PCR to evaluate how the different treatments modified the levels of expression of some key genes involved in oxidative stress and apoptosis. We demonstrated that hydrogen peroxide was able to up-regulate pro-apoptotic genes together with genes involved in DNA repair, such as p21, p53, Bax, GADD45A. The curcumin pre-conditioning showed a gene expression profile similar to the untreated controls and prevented $H_2O_2$ gene expression modifications. These results, taken as whole, indicate a possible role of curcumin as a protective agent against redox insult. These effects seem related mostly to the ability of supply stem cells to resist the hostile microenvironment while preserving their ability to proliferated and engraft. Future in vivo experiment will be performed to sustain these observations, in ischemia/reperfusion mouse model.

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