Effect of rat adipose derived stem cells in a rat model of oxaliplatin-induced neuropathy

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The development of neuropathy is one of the dose limiting side effect of some of the most common chemotherapeutic agents, including platinum drugs. The paucity of specific pharmacologic treatments is linked to the lack of thorough knowledge. In the last few years the scientific community has shown an increasing interest in adult mesenchymal stem cells (MSCs) for the treatment of neuropathic pain. Interestingly, adult MSCs offer a totipotent cellular source for replacing injured neural cells and at the same time represent a source of neuroprotective and anti-inflammatory mediators, opposing the effect of nerve damage. Autologous MSCs can be isolated from bone marrow, but also the stromal vascular cell fraction of adipose tissue is an abundant and easily accessible source of MSCs with phenotypic characteristics and differentiating capabilities similar to bone marrow or embryonal derived MSCs. Furthermore, adipose derived stem cells (ASCs) have a modulatory activity in the neuroinflammatory cascade. The purpose of this study was to evaluate the effect of ASCs in a rat model of oxaliplatin induced neuropathy and a possible mechanism of action. Rat stem cells were isolated from retrosternal fat pads and cultured in an appropriate culture medium. Cells were cytocharacterized by flow cytometry analysis and the immunophenotype was determined: about 85% of isolated ASCs exhibited $CD90^+$ $CD29^+$ $CD45^-$ $CD79a^-$ phenotype, the stemness typical one. Neuropathic pain was induced by repeated oxaliplatin administration (2.4 mg kg^{-1} i.p.). When neuropathy was established, $2x10^6$ ASCs (suspended in 400 μL of DMEM containing 2000 U.I. heparin) were injected into the tail vein of rats. A significant reduction of mechanical hypersensitivity as measured by paw pressure test was observed in ASCs treated rats beginning 1h (54.9 ± 1.8 g vs 43.3 ± 0.0 g of oxaliplatin+DMEM treated rats, $P<0.00000$) and reaching a maximum 6h (66.3 ± 0.3 g vs 43.8 ± 0.9 g, oxaliplatin+DMEM) after the ASCs administration. The effect of ASCs on hypersensitivity began to decrease 48h and disappeared at 72h after ASCs administration. Repeating $2x10^6$ ASCs administration induced a similar reduction of the hypersensitivity, with a similar efficacy trend over time. To evaluate the localization of ASCs in the rat body, the experiment was repeated injecting $2x10^6$ ASCs labelled with 1 μM of the fluorescent probe 5-(and-6-9-(((4-chloromethyl)benzoyl)amino) tetramethylrhodamine. Rats were sacrificed after 48h and tissues were explanted. Before and after ASCs injection, blood was collected and analyzed by flow cytometry to detect the presence of stem cells into bloodstream. One and 3 hours after injection about 0.2% of ASCs of the total blood cell count were found, the percentage gradually decreased and 48 hours after ASCs administration no label cells were found. At this time, ASCs were detected in the liver digested homogenate (0.02% of the total cells). No ASCs were found in the central nervous system and in the lungs. VEGF, EGF and TGF-β were assayed into the plasma collected at different times. EGF and TGF-β were not altered by oxaliplatin or ASCs treatments. On the contrary, VEGF levels increased significantly in oxaliplatin+DMEM as compared to control group. In ASCs treated rats, the VEGF increase was not significantly as compared to control group or oxaliplatin+DMEM treated rats. These data represent a proof of concept of the efficacy of ASCs in oxaliplatin-induced neuropathic pain and a base for the future study of the mechanisms by which these cells may modulate the altered nervous tissue.