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 effects of some of the most common chemotherapeutic agents, including platinum drugs. 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. The purpose of this study was to evaluate the effect of adipose-derived stem cells (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 and reaching a maximum 6h after ASCs administration. The effect of ASCs on hypersensitivity began to decrease 48h and disappeared 72h after ASCs administration. Subsequent ASCs administrations 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. Labelled ASCs were detectable in the bloodstream 1 and 3 hours after injection, the percentage gradually decreased and 48 hours after ASCs administration no cells were found. At this time, ASCs were detected in the liver digested homogenate. No ASCs were found in the central nervous system and in the lungs. VEGF, EGF and TGF-β were assayed. EGF and TGF-β were not altered by oxaliplatin or ASCs treatments. On the contrary, VEGF concentration significantly increased in oxaliplatin-treated rats in comparison to the control group, suggesting a possible implication of VEGF in the development of neuropathic pain. This hypothesis was confirmed by the anti-neuropathic effect induced by acute i.p. administration of the VEGF-antibody bevacizumab at the doses of 1, 5 and 15 mg kg^{-1} in oxaliplatin treated rats. Moreover, the pain-related VEGF-A spliced variant VEGF 165b was significantly modulated by oxaliplatin and ASCs treatments as evaluated in central and peripheral nervous tissues by western blot analysis. Oxaliplatin treatment increased VEGF 165b expression in spinal cord and PAG while in DRG, cortex and thalamus it did not influenced protein levels with respect to control group. ASCs reduced the oxaliplatin-dependent VEGF 165b increase in spinal cord. These data represent a proof of concept of the efficacy of ASCs in oxaliplatin-induced neuropathic pain and suggest a possible pharmacodynamics mechanisms.