

## **VEGF INVOLVEMENT IN THE OXALIPLATIN-INDUCED NEUROPATHY IN RATS: EFFECT OF ADIPOSE STEM CELLS.**

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In particular, in our research we detected an antineuropathic effect of adult rat adipose stromal stem cells (RASCs) in a rat model of neuropathic pain induced by repeated administration of oxaliplatin (OXA, 2.4mg/kg for two weeks, four days a week). Among inflammatory cytokines and growth factors measured in blood of neuropathic rats, we detected an increased concentration of vascular endothelial growth factor (VEGF) in blood of OXA-treated rats as compared to control animals. Twenty-four hours after RASCs administration ( $2 \times 10^6$  cells intravenously), the VEGF blood concentration in OXA-treated rats was significantly reduced. Since the VEGF antibody bevacizumab (Avastin®) in a dose-dependent way (1, 5 and 15 mg kg<sup>-1</sup> i.p.) was also able to decrease pain in OXA-neuropathic rats, we reasoned that pain sensitization measured in OXA-treated rats could be dependent on the VEGF increase in plasma and other nervous tissues. On the other hand, plasma concentration can be only a marker of VEGF level in peripheral and central nervous system tissues, thus VEGF level was investigated in root dorsal ganglia and spinal cord.

In several pathological situations, the effect of VEGF is blunted by the soluble form of the VEGF receptor 1 (sFlt1), that acts as a decoy receptor by trapping its ligand. sFlt1 possesses a high affinity for VEGF as well as the membrane bound type1 receptor (VEGFR1). Therefore, we decided to investigate i) the level of VEGF165b in plasma, dorsal root ganglia (DRG) and spinal cord of neuropathic rats and ii) the mechanism(s) by which RASCs might reduce VEGF pro-algesic effect, hypothesizing that RASCs directly or indirectly could increase sFlt1.

RASCs were isolated from retrosternal fat pad; cells were extensively characterized and used at early passages (P1-P2). Dorsal root ganglia (DRG) and spinal cord were obtained from control, OXA-treated and RASCs injected OXA-treated rats 24 h after cell administration and used to determine VEGF and sFlt1 levels by Western blot.

Membrane and cytosolic fractions of control and in vitro VEGF treated RASCs were analyzed for VEGFR1 and sFlt1. In parallel experiments rat endothelial cells (RCEs) were used as positive control since it is well known that RCEs express VEGFR1 and sFlt1.

In plasma and DRG of OXA-treated rats, VEGF165b protein levels were not modified in comparison to control group, whereas in OXA + RASCs levels were decreased as compared to control and OXA groups. In spinal cord of OXA-treated rats VEGF165b was increased; the administration of  $2 \times 10^6$  RASCs significantly decreased protein level.

In plasma of control, OXA-treated and RASCs injected OXA-treated rats, the level of sFlt1 was very similar, while in DRG sFlt1 was higher in OXA treated group than in control and RASCs injected OXA-treated rats. In spinal cord, a small increase in sFlt1 was detected in RASCs injected OXA-

treated rats. The antibody-identified bands at low molecular weight (25kDa and 40kDa) could represent truncated forms of sFlt1.

In RASCs sFlt1 and VEGFR1 are expressed. After treatment with 1ng/ml of VEGF165b, both proteins rapidly decreased (1h), while they expression were restored after 24h. In RCEs as expected sFlt1 and VEGFR1 were well expressed.

Therefore, we concluded that VEGF plays a central role in OXA-induced neuropathy and RASCs analgesic effect could dependent on a reduction or sequestration of the growth factor. Rat adipose stem cells express VEGFR1 and produce the sFlt1; the in vitro stimulation of the RASCs by the VEGF determines a reduction of the cytosolic concentration of the sFlt1, which could be released. If a similar mechanism is active in the in vivo neuropathic model, the binding of sFlt1 with VEGF could explain the antineuropathic effect of RASCs.