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The effect of ozone therapy at the early period of bone healing in an experimental non-fixed fracture model

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Ozone is a chemical compound, consisting of 3 oxygen atoms. Systemic application of ozone leads to delivery of super enriched oxygen at a cellular level and optimizes cell function. This is achieved by activating the red blood cell, immune-competent cells and the enzymatic antioxidants and radical scavengers at a cellular level (1, 2). Aim of this study was to evaluate the effect of ozone therapy at the early period of bone healing in an experimental non-fixed fracture model.

After ethic committee approval, 48 Spraque-Dawley rats were included in the study. After the standard anesthesia induction technique with intraperitoneal (i.p.) ketamine and xylazine (50 mg/kg from each) rats were randomly divided into 2 groups (n:24 for each groups). In both groups, after anesthesia induction open-femoral fracture (ffx) was performed according to the classical technique. After ffx was performed; in Group Ozone, 4 mL of ozone (10 mcg/mL) and in Group Control, 4 mL of medical air were insufflated rectally during 1 min. via an 18 G cannula once a day for 5 consecutive days.

Open femoral fracture (ffx) technique: After shaving right legs, surgical fields were draped under sterile conditions. Vertical lateral incision at femur was followed by muscular blunt dissection. Femur bone was fractured transversally with bone cutter manually at the level of diaphysis. Bone was not fixated. At Day 4, 7 and 13, eight rats from each group were euthanized with decapitation. Femurs were removed. Samples were evaluated immunohistochemically (via expressions of Vascular endothelial growth factor (VEGF), Transforming growth factor (TGF) and Beta catenin (β-catenin)) and histomorphometrically (thickness of periosteum, trabecule's number and thickness, and trabecular, cartilogenous and new bone areas). One-way analysis of ANOVA was used statistical analysis ($p<0.05$).

In histomorphometric analysis, cartilaginous, new bone and trabecular areas were found smaller in ozone ($p<0.001$ for each). Immunohistochemical analysis revealed that ozone resulted in significant increase in VEGF expressions in Day 7 and 13 ($p<0.01$ and $p<0.05$ respectively). Also, TGF expressions are more intense at all time in ozone group ($p<0.01$ for each) β-catenin expression studied as an indicator of regulation of osteoblast were found higher in ozone group at Day 7 and 13. Although control group had better results at histomorphometric analysis at the early period of non-fixated bone fracture; ozone group, showed more intense expressions of TGF and β-catenin. In the light of this data, we can speculate that however at the early period of non-fixated bone fracture, the results for the cartilaginous, new bone and trabecular areas are worse in ozone group, higher levels of VEGF, TGF and β-catenin could have positive impact on the later period of bone healing process.

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