

## LOW VOLTAGE ELECTRICAL STIMULATION CAUSES CYTOKINE EXPRESSION IN THE ANAL SPHINCTER

### Hypothesis / aims of study

Low grade injury has been used as a conditioning injury in various organs to increase angiogenesis, including hypoxia in the heart and brain (1). Electrical stimulation likewise has the potential to encourage homing of mesenchymal and hematopoietic stem cells to the stimulated region to encourage angiogenesis. SDF-1 and MCP-3 are cell signalling cytokines released during inflammation post injury that are homing factors for stem cells (2) demonstrated by our preliminary study.

Electrical stimulation facilitates transduction of integrins, activation of calcium channels and G protein receptors leading to increase protein synthesis (3, 5). Significant cytokine concentrations are important for an effective cellular communication (3), and electrical stimulation can be effective to enhance cytokine production.

Hypothesis: Electrical stimulation causes low grade injury resulting in cytokine upregulation.

The aim of this study was to investigate the effect of low voltage electrical stimulation on cytokine upregulation as a potential conditioning injury to direct stem cells to the anal sphincter.

### Study design, materials and methods

Forty virgin female Sprague Dawley rats were allocated into electrical stimulation group using a needle electrode that was inserted superficially into the anal sphincter. 0.25 mA current, 40 pulses/sec, 100 $\mu$ s were stimulation parameters used in the anal sphincter for 1 and 4 hours based on our preliminary study. Sham controls for each of the durations were created using the same electrode needle insertion to the anal sphincter without stimulation. The rats were euthanized immediately after stimulation or 24 hours later. The anal sphincter was dissected and cytokine analysis was performed using real-time polymerase chain reaction. Expression of stimulation was normalized to expression of corresponding sham group and was shown as fold expression. Data analysis was done using t-test comparison with  $p < 0.05$  for significant difference.

### Results

After 1 hour stimulation, the expression of SDF-1 and MCP-3 increased (fold expression,  $2.5 \pm 0.77$  and  $3.1 \pm 0.93$ , respectively) immediately after stimulation, in contrast 24 hours after stimulation the expression of SDF-1 and MCP-3 was not significantly different, fold expression ( $1.49 \pm 0.16$ ) and ( $1.51 \pm 0.14$ ), respectively. In the group receiving 4 hours stimulation, no significant elevation of expression in SDF-1 or MCP-3 were noticed either immediately ( $1.21 \pm 0.16$  and  $1.29 \pm 0.41$ , respectively) or 24 hours later ( $0.54 \pm 0.16$  and  $0.35 \pm 0.12$ , respectively) (Figure).

### Interpretation of results

Low current ES causes a low grade injury and increases chemokine expression. Chemokine upregulation has been demonstrated after anal sphincter injury. Electrical stimulation can be used as conditioning injury.

### Concluding message

Low voltage electrical stimulation for 1 hour causes up-regulation of cytokine expression and may facilitate stem cell homing and engraftment to the anal sphincter. This could be a therapeutic option if MSC engraftment can be demonstrated in response to electrical stimulation in future studies. The optimal frequency, pulse, and duration are yet to be explored.

Cytokine expression after electrical stimulation of the anal sphincter

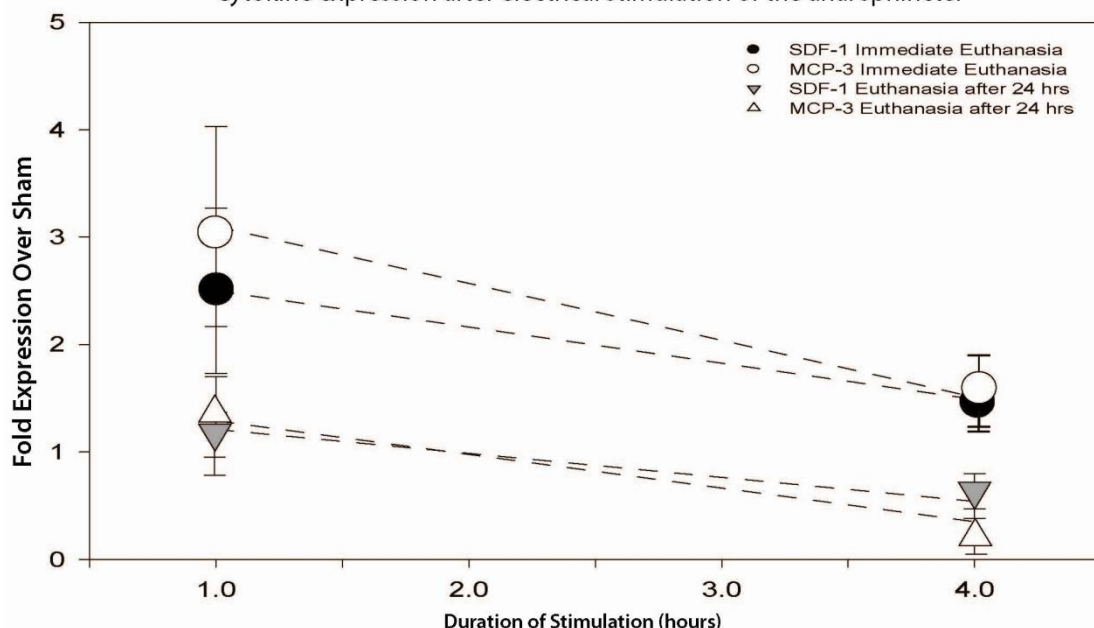


Figure: Cytokine expression showing fold expression of SDF-1 and MCP-3 after 1 and 4 hour stimulation and euthanasia immediately after stimulation and 24 hours after stimulation.

#### References

1. Xing B, Chen H, Zhang M, et al. Ischemic post-conditioning protects brain and reduces inflammation in a rat model of focal cerebral ischemia/reperfusion. J Neurochem 2008; 105(5):1737-45
2. Miller, R. J. and Tran, P. B.: Chemokinetics. Neuron, 47: 621, 2005
3. Miller, M. D. and Krangel, M. S.: Biology and Biochemistry of the Chemokines - A Family of Chemotactic and Inflammatory Cytokines. Critical Reviews in Immunology, 12: 17, 1992

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<b><i>What were the subjects in the study?</i></b>	<b>ANIMAL</b>
<b><i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i></b>	<b>Yes</b>
<b><i>Name of ethics committee</i></b>	<b>INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC)</b>