Journal of Orthopaedic Translation (2016) 7, 112—113

Available online at www.sciencedirect.com

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Session: Spine

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NORMATIVE DATA FOR PARAMETERS OF SAGITTAL SPINAL ALIGNMENT IN 626 HEALTHY SUBJECTS

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Objective: Spino-pelvic sagittal alignment has proven impact on clinical outcome in reconstructive spinal surgery. However, normative data, gender and age related differences are skant. This study aims to establish normative data for parameters of spino-pelvic and spinal sagittal alignment, gender related differences and age-related changes in asymptomatic subjects, using radiographic cross-sectional analysis.
Methods: A total of 626 asymptomatic volunteers were enrolled in this study, including 50 subjects at least for each gender and each decade from 3rd to 8th. Full length biplanar free-standing spine radiographs were obtained in all subjects. Cervical lordosis (CL; C3-7), thoracic kyphosis (TK; T1-12), lumbar lordosis (LL; T12-S1), pelvic incidence (PI), pelvic tilt (PT), sacral slope (SS) and sagittal vertical axis (SVA) were measured.
Results: The average values (degrees) are 4.1 ± 11.7 for CL, 36.0 ± 10.1 for TK, 49.7 ± 11.2 for LL, 53.7 ± 10.9 for PI, 14.5 ± 8.4 for PT, and 39.4 ± 8.0 for SS. Mean SVA is 3.1 ± 12.6 mm. Advancing age caused an increase in CL, PT and SVA, and a decrease in LL and SS. There was a significant gender difference in CL, TK, LL, PI, PT and SVA. From 7th decade to 8th decade, remarkable decrease of LL was seen. In response to LL decrease, decrease of TK and increase of PT might occur to compensate sagittal balance. A large increase of SVA was also seen between 60' and 70', as the compensation to LL decrease was not enough.
Conclusion: Standard values of spinopelvic sagittal alignment were established in each gender and each decade from 20' to 70'. A remarkable change of spinopelvic sagittal alignment was seen from 7th decade to 8th decade in asymptomatic subjects.
http://dx.doi.org/10.1016/j.jot.2016.06.125

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BUPIVACAINE-INDUCED DEATH OF RABBIT INTERVERTEBRAL DISC CELLS INVOLVES ROS-MEDIATED LYSOSOMAL MEMBRANE PERMEABILIZATION

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Introduction: Our previous studies have shown that bupivacaine is toxic to intervertebral disc (IVD) cells in vitro. However, the precise mechanism of bupivacaine-induced IVD cell death is largely unknown.
Subjects and Methods: Both annulus fibrosus (AF) and nucleus pulposus (NP) cells at the second generation maintained in monolayer were exposed to various concentrations of bupivacaine for 60 minutes. Cell viability was evaluated by using CCK-8. Cell death was measured by flow cytometry and the LIVE/DEAD assay. Transmission electronic microscopy (TEM) was used to further characterize the type of cell death after exposure to bupivacaine. The integrity of the lysosomal compartment was evaluated by LysoTracker Red staining. Lysosomal membrane permeabilization (LMP) was confirmed by acridine orange (AO) staining. Moreover, the levels of reactive oxygen species (ROS) were determined with fluorescent probe DCFH-DA.
Results: Bupivacaine treatment induced a dose- and time-dependent cytotoxic effect in rabbit IVD cells. Flow cytometry and fluorescence microscopy showed that the short-term toxic effect of bupivacaine on both AF and NP cells was mainly caused by necrosis. The morphological signs of necrosis such as disruptions in plasma membrane and swollen mitochondria and endoplasmic reticulum were observed under TEM. The key finding of this study was that bupivacaine is able to induce lysosomal membrane rupture, as evidenced by LysoTracker Red staining and AO staining. Finally, we found that bupivacaine resulted in an increase in intracellular ROS, and inhibition of ROS by N-acetyl-L-cysteine (NAC) effectively blocked the bupivacaine-induced LMP and cell death.
Discussion and Conclusion: The results of this in vitro study indicate that a short-term exposure of bupivacaine can cause a rapid cell death, which is primarily due to necrosis, in cultured rabbit IVD cells. In addition, our results show that bupivacaine-induced cell death involves LMP, and ROS is a critical mediator in bupivacaine-induced LMP and cell death. Further investigation regarding in vivo cytotoxicity appears warranted.
http://dx.doi.org/10.1016/j.jot.2016.06.126

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COMPREHENSIVE ANALYSIS OF GENE EXPRESSION IN THE LUMBAR SPINE OF CONGENITAL KYPHOSCOLIOTIC RATS

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Introduction: Congenital scoliosis (CS) is defined as a curvature of the spinal column that is the result of vertebral innate malformation. Currently, there is not the radical cure other than surgical managements to CS. On the other hand, almost nothing is known about the genetic background and key gene for CS. Ishibashi rats (ISR), a model animal representing congenital kyphoscoliosis, are known to show a form extremely similar to human congenital scoliosis. In the previous study, we found unilateral fusions and deformities of primary ossification centers of the lumbar vertebral column in fetal ISR (Seki et al., *Mol. Cell. Biochem.* , 312, 2008). Moreover, we observed that the expression levels of Hox10 and Hox11 paralogs in lumbosacral transitional areas of ISR were extremely low compared with those of normal rats.
Aim: To identify genes responsible for CS, we have analyzed gene expression in ISR comprehensively.
Materials and methods: Five-day-old ISR and Wistar (control) rats were killed and RNA was extracted from the third to fifth lumbar vertebrae where deformities appeared most commonly in ISR. Differences in gene expression profiles between the two strains were analyzed with DNA microarray. Moreover, the gene expression of candidate genes for CS detected with DNA microarray were confirmed by real-time PCR.
Results: In ISR rats, the expression levels of genes related to the bone matrix and retinol metabolism were significant low compared with control rats. Interestingly, the ISR rats showed low levels of gene expression of nerve growth factor receptor. These finding indicate that a low level of gene expression related to bone matrix, retinol metabolism and nerve growth factor signaling induces the formation of abnormal vertebrae. Moreover, ISR rats and its genetic analysis will be useful for elucidation of the onset mechanism of CS.
http://dx.doi.org/10.1016/j.jot.2016.06.127