How do Tamoxifene and Raloxifene effect bipedal scoliotic mice?
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Purpose: To investigate whether progression of experimental scoliosis in bipedal C57Bl6 mice may be by altered by administration of SERMs.

Background: The pathogenesis of Adolescent Idiopathic Scoliosis (AIS) remains unknown, but may be associated with several factors including growth, gender and bipedality. Previous studies demonstrated that a Selective Estrogen Receptor Modulator (SERM) Tamoxifene (TMX) decreases the incidence and progression of spinal curvature in chicken and bipedal mouse models (1,2). Raloxifene (RLX) is another SERM, which is more specific to bone and has fewer side effects compared to TMX. Therefore, we assessed the ability of Raloxifene to reverse scoliotic curves in mice.

Materials and Methods: All procedures were approved by the institutional committee of animal use for research at Hacettepe University, Ankara, Turkey. Ninety, 3-week-old, female C57BL6 mice were made bipedal as described by Machida et al (3) and divided into three groups: 1. TMX, 2. RLX 3. Control. TMX and RLX groups received 10mg/l in drinking water beginning from the 3rd week. Anteroposterior X-rays were obtained at the 20th and 40th weeks. Curves in different locations in the spinal column were identified according to the SRS guidelines and the Cobb angle was measured.

For histomorphological analysis, spinal columns were collected at 20th and 40th weeks (n=4/group) and tissues were fixed overnight at 4°C in 4% paraformaldehyde, decalcified at 4°C in 19% EDTA (pH 7.4) for 10–14 days, then dehydrated in a graded ethanol series and embedded in paraffin. Vertical uniform random sections (10 µm thick) were prepared through the whole block. The lumbar 2nd vertebrae, L2-L3 discs and growth plates on both sides of all specimens were analysed. Every fifteenth section (150 µm) was stained with modified Milligan's Trichrome (TC). Adobe Photoshop was used to capture images from a Leica DM 5000 B light microscope (Leica Microsystems GmbH, Wetzlar, Germany) that was equipped with a camera (Diagnostic Instruments, Inc., Sterling Heights, MI). The number of pixels comprising each tissue component were counted and converted to an estimate of area. Pixels/mm2 was determined using a 1 mm scale bar. Total area was determined by dividing the number of pixels by the number of pixels/mm2. Total corpus volume (TCV), total trabecular volume (TTV), percentage of TTV to TCV (%TTV/TCV), total disc volume (TDV), total nucleus pulposus volume (TNPV) and total growth plate volume (TGPV) were calculated using the equation for a conical frustum: V=1/3h (A1+At1+2At+h), where h is the distance between sections (150µm), and n is total number of sections analyzed for each specimen.

Results:
Radiology: 20th week analysis revealed that lower thoracic curve rate (LTR) was high in RLX group and thoracolumbar rate (TLR) was higher in TMX group compared to control (p=0.029 and p=0.033 respectively). TMX group had higher upper thoracic (UT) curve magnitudes compared to control group (p=0.021). 40th week analysis revealed similar curve rates among groups. RLX group had significantly lower upper and lower thoracic curve magnitudes compared to control group (p=0.001 and p=0.014 respectively). TMX group had also significantly lower UT curve magnitudes compared to control group (p=0.014).

Histology: A representative image used for analysis is shown in Figure 1. TCV (Fig. 2A) of both groups were similar at 20 weeks whereas there was a significant difference between RLX treated and control animals at 40 weeks (p<0.05). No difference was observed between TMX and control groups during these times. The vertebral body of RLX treated mice were smaller than the control group. TTV (Fig. 2B) had the same distribution as TCV at 20 weeks. However, at 40 weeks, TMX-treated animals had higher TTV than but this was not significant. The TTV of RLX animals was almost the same as controls at the 40th week, but the ratio of TTV to TCV (Fig. 2C) in both TMX and RLX groups were greater compared to controls. Both TTV (Fig. 2D) and TNPV (Fig. 2E) were decreased in treated groups at 20 weeks but this was not significant statistically. At 40 weeks, TTV in animals treated with RLX was significantly lower than controls (p<0.05). The growth plates of treated groups were smaller and thinner compared to controls at 20 weeks.

Conclusions: RLX is as effective as TMX in preventing progression of scoliotic curves. Both drugs did not reduce the incidence of scoliosis but the curve magnitudes between groups were reduced. Our findings suggest that the mechanism associated with this is the early maturation of growth plates thereby possible deceleration of the growth rate of the vertebral column. The mechanisms of action of these drugs on the growth plate and the intervertebral disc needs to be explored in further studies.

Significance: Our findings are important as they suggest the possibility of a medical treatment for the prevention of progression in AIS, otherwise treated with cumbersome orthoses and/or surgery with relatively high morbidity.

Figure 1. Histology of control (left), TMX (center), and Rlx (Right) treated animals (Bar=1mm).

Figure 2. Histomorphometry results.

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References: