Conditional Loss of COX-2 in Juvenile and Adult Mice Causes Osteoclast-mediated Spinal Disc Degeneration

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INTRODUCTION: This study posits whether loss of the enzyme COX-2 using a postnatal tamoxifen inducible knockout mouse model results in disc degeneration and spinal osteoporosis, given the important role of COX-2 in prostaglandin production. Previous studies found that endplate ossification is reduced in COX-2 null mice, while treatment with LPS injection in disc puncture models has been shown to induce disc degeneration. To determine whether the prior results showing vertebral unit irregularities observed in COX-2 null mice were related to skeletal development or whether COX-2 was providing a homeostatic function to maintain vertebral unit integrity, we examined vertebral units isolated from 28-week-old mice after conditional deletion of COX-2 at either 4 weeks of age or at 20 weeks of age. The results show that COX-2 is required for vertebral unit integrity and that loss of COX-2 leads to disc degeneration when COX-2 is deleted from either juvenile or adult mice. Additional experiments indicate that the effects of conditional COX-2 deletion on the vertebral unit and disc are osteoclast-mediated.

METHODS: Using Gt(ROSA)26Sor$^{tm1(cre/ERT2)Tyj}$ (ROSA-CreER) mice that are homozygous for a floxed allele of COX-2 (*Ptgs2*^{tm1Hahe}), postnatal induction of COX-2 deletion was induced by tamoxifen (Tm) administration to generate COX-2 cKO^{ROSA} mice under an approved IACUC protocol. Equal numbers of male and female mice were used in all groups. Mice were fed Tm (1g Tm/kg body weight) mixed in a complete diet-gel for 2 weeks to promote cre mediated deletion of COX-2 or mice were fed a complete diet-gel without Tm for the same 2-week period as the placebo control. There were two arms of the study. The first arm of the study used COX-2 cKO^{ROSA} mice to determine the effect of conditional COX-2 deletion on vertebral unit integrity in the context of aging. 12 ROSA-CreER/*Ptgs2*^{tm1} mice and 6 C57BL/6 mice were treated with Tm at 4 weeks of age, while another 12 ROSA-CreER/*Ptgs2*^{tm1} mice and 6 C57BL/6 were treated with Tm at 20 weeks of age. The same number of animals in each genotype were treated with placebo at 4 and 20 weeks of age as controls. The 1st arm mice were euthanized at 28 weeks (60 total mice). In the second arm, 4 groups of 12 ROSA-CreER/*Ptgs2*^{tm1} mice were treated with Tm or placebo at 12 weeks of age and also by subcutaneous injection of zoledronic acid (ZA, 0.06 mg/kg) at 12 and 16 weeks or the vehicle control. The 2nd arm mice were euthanized at 20 weeks (48 total mice). Following euthanasia, spines were dissected, fixed, and then scanned with μCT followed by paraffin embedding, sectioning and data analyses. Experimental outcomes included Thompson scoring for intervertebral disc (IVD) degeneration, μCT analysis of endplate porosity (EP) and not shown osteoclast quantification, collagen fibril analyses, immunohistochemistry, and trabecular porosity (TP).

RESULTS (Arm 1): The Thompson grading of the vertebral unit histology found significant degeneration within the lumbar region in the COX-2 cKO^{ROSA} mice as compared to the placebo controls or the Tm-treated C57BL/6 mice (Table 1). Detailed analysis of the Thompson scores showed that the COX-2 cKO^{ROSA}mice treated at 20 weeks of age had more degeneration than those treated at 4 weeks of age and that degeneration was more severe in male COX-2 cKO^{ROSA} mice than females.

Group	Tm	ZA	Endpoint	IVDD Score	P-value	EP (%)	P-value
C57Bl/6 Arm1	None	None	28 Weeks	9.7 (0.4)	Ns	12 (2)	Ns
	At 20 Wks	None		8.5 (1.6)	Ns	11(2)	Ns
COX-2 cKO^{ROSA} Arm 1	None	None		8.0 (2.3)	Ns	12 (2)	Ns
	At 4 Wks	None		11.3 (0.5)	**<0.0001**	10 (2)	Ns
	None	None		9.8 (2.5)	Ns	11 (2)	Ns
	At 20 Wks	None		15.6 (1.7)	**<0.0001**	22 (4)	**0.005**
COX-2 cKO^{ROSA} Arm 2	None	None	20 Weeks	7.6 (2.0)	Ns	12 (3)	Ns
	At 12 Wks	None		12.0 (1.5)	**<0.0001**	17 (3)	0.02
	None	At 12 Wks		8.2 (2.1)	Ns	9 (2)	Ns
	At 12 Wks	At 12 Wks		7.5 (1.2)	Ns	8 (2)	Ns

Values represent means +/- (std)

RESULTS (Arm 2): Thompson grading of the vertebral units found that ZA treatment prevented vertebral unit degeneration in the COX-2 cKO^{ROSA} mice (Table 1). In both arms of the study, vertebral unit degeneration in the COX-2 cKO^{ROSA} mice was characterized by blood vessel infiltration into the vertebral growth plate and endplate (Figure 1), localized presence of F4/80 macrophages at focal disruptions, and severe disc degeneration associated with osteoclasts within the growth plate, endplate, annulus fibrosus and transition zone. The degenerative changes also corresponded to reduced endplate porosity (Table 1) in COX-2 cKO^{ROSA} mice, which was prevented by ZA treatment.

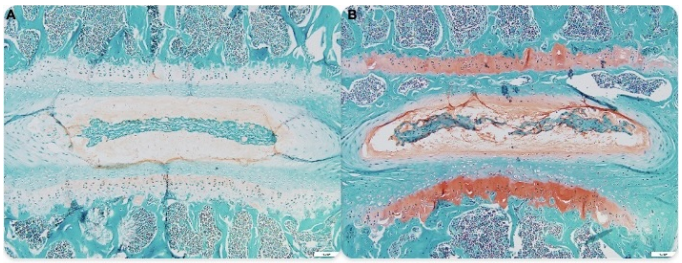

Figure1: Representative 10X histology images of mouse lumbar IVDs at 28 weeks of age. Scale bar is 100 μm. Safranin o-fast green staining is shown for mice treated with either A) placebo, B) tamoxifen at 20 weeks of age for 2 weeks and aged to 28 weeks.

DISCUSSION: In a prior study, Ding et al. (PMC5883113) found that genetic ablation of COX-2 in mice (COX-2 KO) lead to degenerative changes in the intervertebral disc (IVD). Here, we extended those observations by conditionally deleting COX-2 in juvenile and adult mice to differentiate a developmental effect of COX-2 on the IVD versus a homeostatic function in preserving the IVD. We found that post-natal deletion of COX-2 was associated with loss of transition zone matrix resulting in IVD degeneration and increased endplate porosity. The observed IVD degeneration in the COX-2 cKO^{ROSA} mice was more severe in the mice induced to undergo COX-2 deletion at 20 weeks of age as opposed to 4 weeks of age. Thus, the effects of COX-2 deletion on vertebral unit integrity can occur after skeletal maturation in the mice. This would suggest that therapeutic inhibition of COX-2 may lead to or exacerbate degeneration of the vertebral unit, including IVD degeneration. In addition, we were able to show that ZA treatment prevented IVD degeneration in the COX-2 cKO^{ROSA} mice, indicating that the degenerative effects of lost COX-2 activity are mediated by osteoclasts.

SIGNIFICANCE/CLINICAL RELEVANCE: At some point, back pain affects 80% of adults and is often associated with degeneration of one or more IVDs. These novel results indicate that inhibiting COX-2 with NSAIDs or other drugs may exacerbate disc degeneration.

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