In vivo pharmacodynamics of Peniel 2K, a peptide that regulates TGF-beta signaling on disc degeneration

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Background & Significance

- For the biological treatment of degenerative disc disease (DDD), the stimulation of matrix synthesis by various growth factors has been proposed.
- Transforming growth factor-β (TGF-β) is a multifunctional regulator of cellular proliferation, differentiation and extracellular matrix (ECM) production.

  - Anabolic effects of TGF-β on proteoglycan synthesis in canine disc cells. Thompson et al. 1991
  - TGF-β stimulates proliferation of human annulus fibrosus cells. Gruber et al. 1997
  - Overexpression of TGF-β had deleterious effect on degenerated cartilage tissue via increase in MMP-13 and aggrecanase-1. Blaney Davidson et al. 2009
  - Strong expression of TGF-βs and their receptors in disc tissues from patients with painful DDD, and levels of TGF-β and cytokine expression were higher in patients with DDD than in those with herniated disc. Peng et al. 2006 Lee et al. 2009
• The regulation of TGF-β expression is important in the degenerative processes in IVDs.

• A novel peptide named Peniel 2000 (P2K); YH14618 with a binding activity to TGF-β1 was explored by a knowledge-based in silico drug discovery strategy.

• In a previous study, we demonstrated that P2K showed an anabolic effect on bovine IVD cells and degenerated disc using rabbit model. Kwon et al. 2013

• The purpose of this study was to investigate in vivo pharmacodynamics of P2K in rabbit degenerated disc and determines the maximal effective dose in in vivo.
Materials and Methods

• In 15 New Zealand white rabbits, disc degeneration was induced by percutaneous annular punctures (Kwon, 2013) and confirmed 4 weeks after the puncture by the decrease in disc height in X ray.
• Twelve weeks after treatment (16 weeks from initial puncture), the regenerative activity in the disc was examined by X-ray radiography, magnetic resonance imaging (MRI), and histological analyses with H&E and Safranin O stains.
• Five treatment groups according to P2K dose
  ■ 0µg (5% lactose as a solvent)/ 3µg/ 10µg/ 30µg/ 60µg
• Longitudinal X-ray data in each treatment group were analyzed with repeated measures analysis of variance (ANOVA) with multiple comparisons, adjusted by Bonferroni method.
• The difference of % DHI between groups at 16 weeks was analyzed with analysis of covariance (ANCOVA) to adjust for pre-existing difference at 4 weeks. The Kruskal-Wallis test was used to analyze the MRI and the histological grades in each group. The Bonferroni correction was used for multiple comparisons.
DH = Disc Height, LB = Lower Body Height, UB = Upper Body Height

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DHI = \frac{2(DH1 + DH2 + DH3)}{(LB1 + LB2 + LB3) + (UB1 + UB2 + UB3)}
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% DHI = (post-injected DHI / pre-injected DHI) x 100
By RMANOVA; 10µg group, $P=0.035$. 30µg group, $P<0.001$.
By ANCOVA with pairwise comparison between lactose and P2K groups; 30µg group, $P=0.001$. 
T2-weighted sagittal MR

Lactose

3 ug

10 ug

30 ug

60 ug

MRI grades

* P<0.05 vs lactose group
Normal

Lactose

P2K 3 ug

P2K 10 ug

P2K 30 ug

P2K 60 ug

Ensoltek

Histological grades

Histology

* P<0.05 vs lactose group
Discussion

- TGF-β can signal via two different type I receptors (ALK1 vs ALK5) with opposite effects

- Whereas activation of ALK5 by TGF-β results in inhibition of migration and proliferation, TGF-β-induced ALK1 activation results in increased migration and proliferation of endothelial cells. *Goumans et al.* 2002

- ALK1 dependent Smad1/5/8 signaling inhibits TGF-β/ALK5-dependent Smad3-driven transcriptional activity and ECM production in human chondrocytes. *Finnson et al.* 2008

- Besides the phosphorylation of Smad 2/3, our previous results showed that TGF-β1 increased Smad 1/5/8 phosphorylation in bovine disc cells. P2K preferentially inhibited phosphorylated Smad 1/5/8. *Kwon et al.* 2013
TGF-β1

Smad2
Smad1/5/8

Synthesis of ECM components
Disc degeneration

(Degenerative condition)

TGF-β1

Smad2
Smad1/5/8

Synthesis of ECM components
Disc regeneration

(P2K treatment)

TGF-β1

Smad2
Smad1/5/8

↓ Synthesis of ECM components
Disc regeneration

(P2K over-treatment)
Conclusion

• Regulated inhibition of TGF-β1 signaling, mainly *Smad 1/5/8 pathway*, has an important role in the regeneration of degenerated discs.
• The results show that range of maximal effective dose of P2K would be 10 to 30ug /disc.
• Based on our results to show the range of maximal effects, the concentration of P2K should be adjusted according to the intra-discal concentration of TGF- β1 and the degree of IVD degeneration.

Disclosure

• This work was supported by Yuhan Corporation, Korea
• YH14618 is a code name of Peniel 2K at Yuhan Corporation