DOSE-RELATED CELLULAR EFFECTS OF PLATELET-DERIVED GROWTH FACTOR-BB VARY BETWEEN DIFFERENT TYPES OF RABBIT TENDONS IN VITRO

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Introduction: Healing of sutured flexor tendons within the intrasynovial region of the hand may be site or donor specific. During tissue repair, growth factors including platelet-derived growth factor (PDGF) induce specific biological responses, such as cell proliferation and migration, matrix synthesis, angiogenesis and release of growth factors (Pierce et al. 1989). Although PDGF-BB is known to be the strong stimulator on cell proliferation in a variety of tissues and cell types in vitro (Lepistö et al. 1992), its stimulatory effect on matrix synthesis has been considered less prominent (Bartold 1993). The stimulatory effects of Insulin-like growth factor-I (IGF-I) on cell proliferation and matrix synthesis have been demonstrated to be differentiated between various types of tendons in vitro, suggesting capability of similar effects of PDGF-BB (Abrahamsson et al. 1996). The purpose of this study is to compare the stimulatory effects of PDGF-BB on cell proliferation and matrix synthesis between different regions and types of rabbit tendons in vitro.

Methods
Six mixed gender Swedish Loop rabbits, weighing 2.2-2.8 kg, were used. One segment from the proximal region of the deep flexor tendon at the level of the metatarsophalangeal joint (FDPp) and one segment from the intermediate region at the level of the proximal phalanx (FDPi) were each divided and split into 6 mm-long segments. The peroneal tendons (PER) were pulled out of paratenon and divided into sixteen 10 mm-long segments. These segments were rinsed, placed in culture plates, and incubated in medium MCDB 105, supplemented with gentamycin (50 µg/mL), ascorbic acid (50 µg/mL) and bovine serum albumin (1 mg/mL), for 24 h at 37°C in a water-saturated atmosphere of 2% CO2. On the second day, the supplemented medium was replaced with fresh medium. Recombinant human Plackett derived growth factor-BB (PDGF-BB) was added (0, 0.1, 0.3, 1.0, 3.0, 10.0, 30.0, 100.0 ng/mL) to two sets of eight groups of tendon segments, respectively. On the third day, the procedure was repeated and one set of 144 tendon segments were labeled for 24 h with 3H-thymidine (10 µCi/mL) for detecting DNA synthesis, while the other set of tendon segments were labeled with 3H-diamidine (10 µCi/mL) for detecting DNA synthesis. On the fourth day, the segments were rinsed once and chased incubated twice for 30 minutes in supplemented medium complemented with L-proline (50 µg/mL) or thymidine (50 µg/mL). The segments were frozen, lyophilized, weighed and incorporation rates were determined (Abrahamsson et al. 1989).

Statistical Analysis
Emax (estimated maximum response; %) refers to the maximum response of an added factor (max; dpm/mg dwt) given as percentage of the response without the factor (con; dpm/mg dwt); Emax = max/con x 100. LogEd50 refers to the logarithm of the estimated dose of an added factor eliciting the half-maximal effect (½max - con) / 2 + con) and was calculated from the equation of the linear regression line. Unless stated otherwise, the results are presented as mean ± standard deviation (SD). Neuman-Keul’s test (ANOVA) was used when comparing multiple. A value of p<0.05 was considered significant.

Results
The effects of PDGF-BB on synthesis of matrix components, including proteoglycan, collagen, and noncollagen protein, were all dose dependent between 0.1-30 or 0.1-100 ng/mL, in the three types of tendon segments, with the only exception of the synthesis of collagen in FDPp between 0.1-3 ng/mL. The effects of PDGF-BB on DNA synthesis in FDPi and FDPp were dose dependent between 0.1-30 and 0.1-100 ng/mL, respectively, and for PER segments between 0.1-10 ng/mL (Fig. 1).

The level of Emax, representing the quantitative response of PDGF-BB, on the rate of synthesis of all three matrix components did not differ significantly between the three types of tendon segments, and neither did for DNA synthesis.

The levels of LogEd50 of synthesis of collagen and noncollagen protein, which allows to calculate the potency of the stimulation by PDGF-BB, were significantly lower in FDPp than PEr (-1.68 ± 1.28 and 1.15 ± 0.24, respectively; p<0.05) or FDPi and PEr, (0.64 ± 0.31, 1.01 ± 0.32 and 1.23 ± 0.10, respectively; p<0.05). The level of LogEd50 of DNA synthesis in FDPp was significantly higher than in FDPi (1.31 ± 0.45 and 0.61 ± 0.47, respectively; p<0.05).

Discussion
Our results demonstrate that PDGF-BB dose-dependently stimulates matrix synthesis and cell proliferation in intermediate and proximal intrasynovial flexor and extrasyonovial peroneal tendon segments of rabbits in vitro. We observed that the rate of matrix synthesis and cell proliferation, represented by quantitative response of PDGF-BB (Emax), did not differ between the three types of tendon segments. In contrast, the potency of PDGF-BB (calculated from LogEd50) stimulating synthesis of matrix components and cell proliferation varied between different types of tendon segments. The highest potency of synthesis of collagen and noncollagen protein and lowest potency of cell proliferation were observed in proximal intrasynovial tendon segments. These results correlate with previous observations that fibrocartilaginous-like regions within proximal intrasyonovial flexor tendons and suggest that the sensitivity to PDGF-BB differs between various regions and types of tendon segments (Okuda et al. 1987, Abrahamsson et al. 1996).

As we observed only minor stimulatory effects of PDGF on collagen synthesis in proximal intrasynovial flexor tendon segments, our results also suggest that besides being a potent mitogen, PDGF may also modulate matrix synthesis in different rabbit tendon segments in vitro. PDGF induces synthesis of other growth factors including IGF-I and regulate the presence of other receptors (Pierce et al. 1991). These observations support the hypothesis that PDGF may be a key growth factor and stimulant during the early stages of tendon healing, collaborating with other growth factors. In addition, the results indicate the possibility of clinical applications of PDGF enhancing intrinsic tendon metabolism during tendon surgery.

References

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