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Short Communication

Pleiotrophin promotes capillary-like sprouting from senescent aortic rings

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ABSTRACT

Background: Pleiotrophin (PTN) is a heparin-binding growth factor involved in angiogenesis during development and tumor growth. Plasmid therapy with PTN also induces angiogenesis after myocardial infarction. During aging, angiogenesis is impaired and we therefore examined whether a growth factor therapy with PTN is able to restore neovascularization.
Methods: We evaluated the PTN effects on capillary-like endothelial sprouting in adult ($n = 10$) and senescent ($n = 10$) rats, using an *ex vivo* model of explanted aortic segments in culture. Freshly cut thoracic aortic rings from 3 and 24 month old (mo) rats (both $n = 12$) were cultured in a 3-dimensional collagen matrix with or without addition of recombinant human PTN (2.5–250 ng/ml) or Vascular Endothelial Growth Factor-165 (VEGF) (1–100 ng/ml) and the length of developed capillary network was quantified at day 3 and 6 by image analysis.
Results: After 6 days of culture, capillary-like tube formation was lower in control conditions in 24 mo aortic rings than in 3 mo rings. Addition of PTN increased dose-dependently the length of capillary-like tube formation in both 3 and 24 mo rings ($P < 0.001$ and $P < 0.001$ respectively). Age-associated impairment of capillary-like tube formation had been successfully restored in senescent aortic segments by PTN treatment. PTN induced development of capillary network similar to that observed with VEGF therapy with doses equal or superior to 10 ng/ml.
Conclusion: PTN is able to induce *ex vivo* angiogenesis during aging and might be a new promising therapy to induce neovascularization in aged tissues as well as after age-associated cardiac, hindlimb or cerebral ischemia.

1. Introduction

Aging is associated with a severe impairment of angiogenesis i.e. the formation of new blood vessels from a pre-existent vasculature, resulting from alteration of expression and/or secretion of angiogenic growth factors [1,2]. Vascular Endothelial Growth Factor (VEGF) demonstrated efficacy in promoting coronary angiogenesis and arteriogenesis in tissues from aged animals [2] and in some human trials [3] but VEGF also had adverse effects such as vascular leakage [4].

Pleiotrophin (PTN) is a heparin-binding growth factor involved in nervous system development and in tumor angiogenesis and growth. While its mitogenic activity is well established [5], its potential beneficial proangiogenic effect to induce therapeutic neovascularization remains largely unexplored [6,7] but may be of interest, especially during aging. Indeed, in adult rodents, the injection of PTN plasmid DNA induced both capillary and arteriole growth after myocardial infarction [6] and the injection of PTN-expressing monocytes improved perfusion after hind-limb ischemia [7].

Using the aortic ring assay, a model extensively used to study the effects of different agents on the development of branching capillary-like structures [8], we then evaluated the proangiogenic effects of PTN protein on capillary-like tube formation in cultured aortic from 3 and 24 month old (mo) rats.

2. Materials and methods

2.1. Animals and hemodynamics

Investigations were conducted with 3 (adult; $n = 10$) and 24 (senescent i.e. 50% mortality in the population; $n = 10$) mo male

Abbreviations: VEGF, vascular endothelial growth factor; PTN, pleiotrophin; ALK, N-syndecan anaplastic lymphoma kinase; RPTPβ/ζ, receptor protein tyrosine phosphatase β/ζ.

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Wistar rats (Charles River Laboratories, France). All *in vivo* experiments, approved by the appropriate French committee in charge of animal experimentation (Direction Départementale de la Protection des Populations, Val de Marne, France), were conducted in compliance with the international laws and policies (European Communities Council Directive of 24 November 1986, 86/609/EEC). Aortic systolic and diastolic blood pressures were measured using a Mikro-Tip® Pressure catheter (Millar Instruments Inc, HSE, Germany) in anesthetized 3 and 24 mo rats, as previously reported [2].

2.2. Production of VEGF and PTN proteins

Recombinant human $VEGF_{165}$ protein was produced in Sf9 insect cells with the recombinant baculovirus expression system and recombinant human PTN was produced by transformation of the expression vector pet-H8 into *Escherichia Coli*, as previously reported [9]. VEGF and PTN protein purification were performed by cation exchange and heparin-affinity chromatography [9]. Biological activity of produced growth factors was tested on proliferation of Human Umbilical Vein Endothelial Cells (HUVECs were obtained from Lonza, Switzerland, catalog number C2519A).

2.3. Aortic ring assay

Aortic rings were cultured in 3-dimensional collagen gels, as previously reported [2]. Three and 24 mo rat thoracic aortas, cut in 1–2 mm-long aortic rings to obtain between 12 and 14 rings per aorta, were embedded in 2 ml of collagen gel and maintained at 37 °C in DMEM + 10% (v/v) fetal calf serum (PAA) + 50 μg/ml Gentamycine (Invitrogen, France). At day 0 and 3, saline solution (control) or different concentrations of VEGF (1–100 ng/ml) or PTN (2.5–250 ng/ml) were added to the culture medium of 3 ($n = 12$) and 24 ($n = 12$) mo rings. Rings were photographied at day 3 and 6 by phase contrast microscopy. The measurement of maximal microvessel length from the aortic explants was processed using image analysis (MetaMorph 7.0 software).

2.4. Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) and subsequently by Newman–Keuls test for post-hoc comparisons. A $P < 0.05$ was considered significant.

3. Results and discussion

Aortic rings embedded in collagen gels produce angiogenic outgrowths (Fig. 1A) as previously reported [8]. In control conditions, the capillary network, similarly but poorly developed at day 3 at both ages, was strongly reduced in 24 mo rings (−25.2% vs 3 mo rings; Fig. 2) at day 6 (Fig. 2). This age-associated impairment of capacity of capillary-like tube formation in 24 mo rings did not result from hypertension during aging since aortic systolic

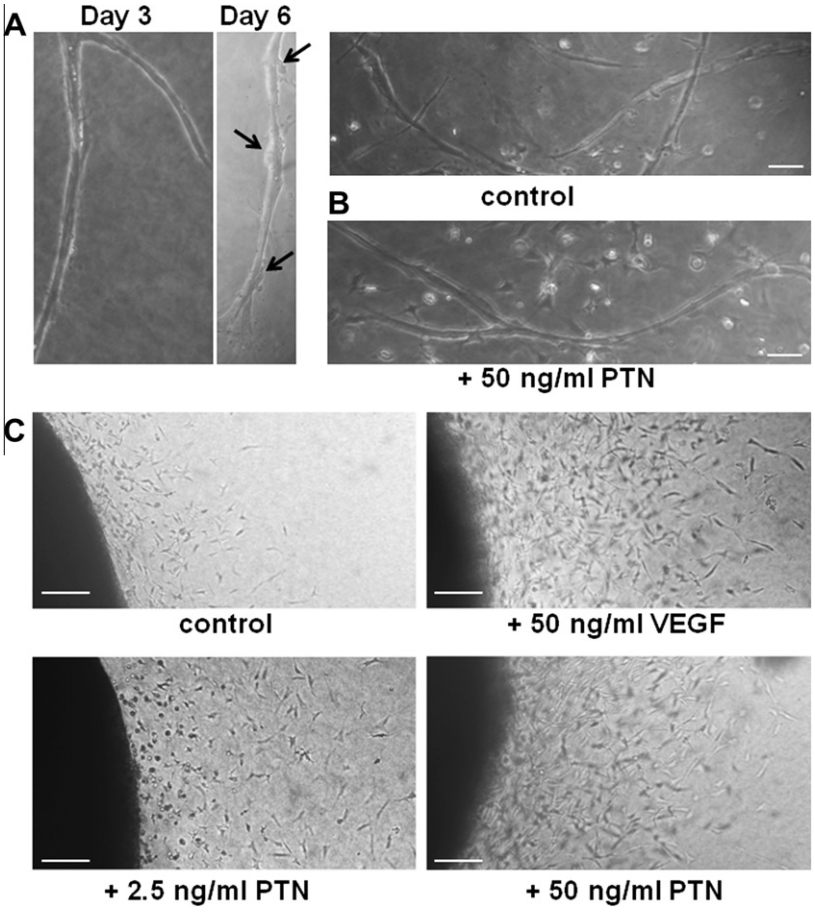

Fig. 1. Capillary outgrowth in aortic rings after PTN and VEGF administration. Panel A: High magnification of typical formed microvessels, from 3 mo ring in control condition, first constituted by endothelial cells forming a branching tube as soon as day 3 of culture and then surrounded by mural cells (arrows) at day 6 of culture. Panel B: Capillary-like tubes with branched sprouts from 24 mo rings after 6 days of culture in control conditions and after addition of 50 ng/ml PTN. Bar: 100 μm. Panel C: Phase-contrast images of 24 mo aortic rings embedded in type I collagen in control conditions and after addition of 50 ng/ml VEGF or 2.5 and 50 ng/ml PTN. Bar: 200 μm.