

Pregnancy derived products for treatment of perinatal brain injuries

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Chapter 8
Summary

Pregnancy derived products for treatment of perinatal brain injuries

Introduction

Perinatal brain injuries affect both preterm and term born infants and successful therapies are lacking. In the last decade, new therapeutic strategies have emerged for perinatal brain injuries with embryo and placental derived products such as mesenchymal stem/stromal cells (MSCs), Estradiol, and PreImplantation Factor (PIF) being of special interest. The objective of this translational research was to establish (1) the feasibility and protective capacity of Wharton`s Jelly derived MSCs (WJ-MSCs), (2) the potential of 17 β -Estradiol supplementation, (3) the neuroprotective effects of synthetic PreImplantation Factor (sPIF) in murine models of perinatal brain injury. The underlying mechanisms were dissected as well.

Methods

We evaluated the neuroprotective potential of embryo and placenta derived products in vitro using neuronal and immune cells and in vivo using rat models of perinatal brain injury. To test WJ-MSCs (1) potential we developed a model of stereotactic intracerebral injections in neonatal rats and subsequently tested WJ-MSCs in an immature brain injury model. To screen for potential signaling pathways we used well defined motif antibodies and performed Western Blots. 17 β -Estradiol (2) and sPIF (3) were tested in neonatal brain injury models and the underlying mechanisms (3) were dissected in vitro. Functional tests were performed (1) and all brains were assessed by histology.

Results

WJ-MSCs (1) after stereotactic intracerebral transplantation migrate throughout the ventricle system and home in the brain. WJ-MSCs reduce injury-mediated myelination loss and astroglial activation while preserving proper functional behavior of the animals. Importantly these observed protective effects were mediated in part by targeting crucial signaling pathways involved in cell cycle regulation and apoptosis. Following brain injury WJ-MSCs modulate cyclin-dependent kinase (CDK), Protein kinase B (Akt), and 14-3-3 binding protein partners.

17 β -Estradiol (2) reduces macroscopically and microscopically brain damage after injury. Treatment results in restored cerebral volume and significantly reduced number of apoptotic cells.

Subcutaneously injected sPIF (3) co-localized with both neurons and glia. sPIF abrogated neuronal loss and glial activation after injury while shifting microglia anti-inflammatory state. Further, sPIF restored cortical architecture and neuronal morphology while reducing the number of apoptotic cells. Mechanistically, sPIF reduced the biogenesis of let-7, which in the extracellular environment causes cell death. sPIF decreased production of let-7 by destabilizing KSRP, a key microRNA processing protein, in a TLR4/PI3K/Akt-dependent manner. Additionally, in a TLR4-dependent fashion, sPIF modulated PKA/PKC signaling. sPIF increased phosphorylation of neuroprotective substrates GAP-43, CREB, and BAD, which in turn stimulated expression of downstream genes *Gap43*, *Bdnf* and *Bcl2*.

Conclusion

Pregnancy derived products such as Wharton`s Jelly derived MSCs, 17β -Estradiol, and synthetic PreImplantation Factor protect the immature brain after perinatal injury. These effects are partially mediated by modulating non-coding RNAs and cell fate signaling pathways.

Chapter 9

Nederlandse samenvatting

Summary in Dutch

Zwangerschaps afgeleide producten voor de behandeling van perinataal hersenletsel

Introductie

Perinataal hersenletsel heeft invloed op zowel prenatale alsook a temre geboren kinderen en succesvolle therapieën ontbreken. In de laatste 10 jaar, zijn nieuwe therapeutische strategieën ontstaan voor perinataal hersenletsel bij embryo's en zijn placenta afgeleide producten als mesenchymale stam-/bindweefsel cellen (MSC's), Estradiol en PreImplantation Factor (PIF) van speciaal belang. Het doel van dit translationeel onderzoek was om de haalbaarheid en de beschermende capaciteit van Wharton Jelly afgeleide MSC's, het potentieel van 17 β -Estradiol suppletie, en de neuroprotectieve effecten van synthetische PIF (sPIF) in muismodellen van prenatale hersenletsel te behalen. De onderliggende mechanismen werden ook ontleed.

Methoden

We evalueerden het neuroprotectieve vermogen van embryo en placenta afgeleide producten in vitro met behulp van neurale en immuuncellen en in vivo in ratmodellen van prenatale hersenbeschadiging. Om WJ-MSC's potentieel te testen hebben we een model van stereotactische intracerebrale injecties in neonatale ratten en vervolgens WJ-MSC's getest in onvolgroeide hersenletsel model ontwikkeld. Om te screenen op mogelijke signaalwegen gebruikten we goed gedefinieerde motief antilichamen en uitgevoerd Western Blots. 17 β -Estradiol en sPIF werden getest in neonatale hersenletsel modellen en de onderliggende mechanismen werden ontleed in vitro. Functionele test werden uitgevoerd en alle hersenen werden beoordeeld door histologie.

Resultaten

WJ-MSC's na stereotactische intracerebrale transplantatie migreren gedurende de ventrikel systeem en thuis in de hersenen. WJ-MSC's verminderen letsel gemedieerde myelinisatie verlies en astroglial activation met behoud van een goede functionele gedrag van de dieren. Belangrijker werden deze waargenomen beschermende effecten gedeeltelijk gemedieerd door zich te richten cruciale signaalwegen betrokken bij de regulering van de celcyclus en apoptose. Na hersenletsel WJ-MSC moduleren cycline-afhankelijke kinase (CDK), proteïne kinase B (Akt) en bindend 14-3-3 eiwit partners. 17 β -Estradiol vermindert macroscopisch en microscopisch hersenschade na een blessure. Behandeling leidt gerestaureerd hersenvolume en significant verminderd aantal apoptotische cellen. Subcutaan sPIF co-gelocaliseerd met zowel neuronen en glia. sPIF afgeschaft neuronaal verlies en gliacellen activering na een blessure, terwijl er verschuiving is van microglia anti-inflammatoire toestand. Verder sPIF herstelde corticale architectuur en neuronale morfologie, gedurende het verminderen van het aantal apoptotische cellen. Mechanistisch sPIF verminderde de biogenese van let-7, die in het extracellulaire milieu celdood veroorzaakt. sPIF verminderde productie van let-7 door destabiliseren KRSP, een belangrijke microRNA processing eiwit, in een TLR4 / PI3K / Akt-afhankelijke manier. Bovendien, in een TLR4-afhankelijke wijze, sPIF gemoduleerde PKA / PKC signalering, sPIF verhoogde fosforylering van neuroprotectieve substraten GAP-43, CREB en BAD, die op hun beurt de expressie stimuleerde van stroomafwaarts genen Gap43, Bdnf en Bcl2.

Conclusie

Zwangerschap afgeleide producten, zoals Wharton's Jelly afgeleide MSC's, 17 β -Estradiol, en synthetische PreImplantation Factor beschermen van de onvolgroeide hersenen na perinataal letsel. Deze effecten worden gedeeltelijk gemedieerd door modulerende niet-coderende RNA's en mobiele bestemde signaalroutes.