Neuropeptides and neurotransmitters in keratocytes – importance in corneal wound healing processes

Marta Słoniecka

Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av medicine doktorsexamen framläggs till offentligt försvar i Sal KB3B1, KBC-huset, Måndagen den 21 december, kl. 9:00. Avhandlingen kommer att försvaras på engelska.

Fakultetsopponent: Professor May Griffith, Institutionen för klinisk och experimentell medicin, Avdelning för cellbiologi, Linköpings universitet, Linköping, Sverige
Background: The cornea is the outermost transparent layer of the eye and it is responsible for the majority of the eye’s total focusing power. Keratocytes are the resident cells of the corneal stroma and their function is to produce extracellular matrix components and to take part in corneal healing after injury, which may occur due to trauma, infection or surgery. The process of corneal wound healing is complex. Shortly, keratocytes adjacent to the corneal wound undergo apoptosis and remaining cells start the process of proliferation and migration in order to close the wound. Next, an influx of inflammatory cells such as macrophages and neutrophils occurs in order to clear the cornea from cellular debris. The final stage of the healing process restores the quiescent state of keratocytes and remodels any disordered extracellular matrix components, leading to a healthy, transparent cornea. However, when the process of corneal wound healing is incomplete or disturbed, corneal scarring may occur, which can lead to significantly impaired vision. Despite extensive research on corneal wound healing, corneal scarring remains a major cause of preventable blindness. The healing process is dependent on various cytokines and growth factors. However, it is possible that other signal substances are involved. Substance P (SP) is a neuropeptide well known for its role in pain perception. It has been shown that SP can also be produced by non-neuronal cells, including cells of the cornea, and that it can have vast effects on physiological functions, including immune cell activity, and cellular processes, such as cell migration, proliferation, and production of proinflammatory cytokines. Similarly, acetylcholine (ACh), a classical neurotransmitter, has also been reported to be produced by non-neuronal cells, including corneal epithelium, and to be involved in cell proliferation, angiogenesis, cell migration, apoptosis, and collagen gene expression. In the studies of this thesis, it is hypothesized that neuropeptides and neurotransmitters are produced by human keratocytes and that this production is increased in response to corneal injury. Moreover, it is hypothesized that the non-neuronal SP and ACh produced by injured keratocytes participate in corneal wound healing by enhancing keratocyte migration and proliferation, and/or by decreasing keratocyte apoptosis. The aims of this thesis project were to test these hypotheses and to study the underlying inter- and intracellular mechanisms of the effects of SP and ACh on keratocytes.

Results: Cultured primary cells of the human corneal stroma expressed keratocyte markers (keratocan, lumican, CD34, and ALDH), the tachykinins SP and NKA, catecholamines (adrenaline, noradrenaline and dopamine), ACh, and glutamate. Moreover, the cells expressed neurokinin-1 and -2 receptors (NK-1R and NK-2R), dopamine receptor D2, muscarinic ACh receptors (mAChRs) M1, M3, M4 and M5, and NDMAR1 glutamate receptor. Significant differences were observed between expression profiles in cultured keratocytes obtained from central and peripheral cornea. Such differences could also be seen between keratocytes cultured under various serum concentrations. Expression and secretion of SP in cultured keratocytes was increased in response to injury in vitro. SP enhanced migration of cultured keratocytes through stimulation of its preferred receptor, the NK-1R, and activation of the phosphatidylinositide 3-kinase and Rac1/RhoA pathway and subsequent actin cytoskeleton reorganization and formation of focal adhesion points. Moreover, SP stimulation led to upregulated expression of the proinflammatory and chemotactic cytokine interleukin-8 (IL-8), which also contributed significantly to SP-enhanced keratocyte migration and to attracting neutrophils. ACh enhanced keratocyte proliferation in vitro at low concentrations and this stimulation was mediated through activation of mAChRs and activation of MAPK signalling. Moreover, ACh stimulation led to upregulation of two proliferation markers: PCNA and Ki-67. ACh was also able to protect cultured keratocytes from Fas-induced apoptosis, even at low concentrations. Activation of mAChRs was necessary for this latter process to occur. ACh reduced caspases 3/7 activation in Fas-treated keratocytes. Inhibition of the PKB/Akt pathway revealed that its activation is essential for mediating the anti-apoptotic effect of ACh in keratocytes.

Conclusions: This thesis shows that human keratocytes express an array of neuropeptides (SP, NKA) and neurotransmitters (ACh, adrenaline, noradrenaline, dopamine and glutamate), and their receptors, and that stimulation of NK-1R by SP and stimulation of mAChRs by ACh lead to keratocyte cellular processes that are known to be involved in corneal wound healing. Specifically, SP enhances keratocyte migration through upregulation of IL-8, ACh enhances keratocyte proliferation through activation of the MAPK signalling pathway, and ACh is able to protect keratocytes from apoptosis by activation of the PKB/Akt pathway. Taken together, these findings suggest that both SP and ACh, if entered at the proper stage, could be beneficial for corneal wound healing.

Keywords
substance P, acetylcholine, migration, proliferation, apoptosis, corneal stroma

Language   ISBN       ISSN       Number of pages
English   978-91-7601-385-4   0346-6612   56 + 4 papers