Cajal–like interstitial cells as a novel target in detrusor overactivity treatment: true or myth?

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Introduction. The Cajal–like intestitial cells (ICCs) act as a pacemaker and are responsible for generating smooth muscle activity in the gastrointestinal tract (GI). Interstitial cells that resemble ICCs in the GI have been identified in the urinary bladder.
Materials and methods. This review is based on a systemic literature research. The medline/pubmed, scopus, embase, and Web of Science databases were browsed in order to identify original and review articles, as well as editorials relating to cajal–like cells, urinary bladder, detrusor overactivity, overactive bladder, glivec, etc. The controlled vocabulary of the Medical Subject Headings (MeSH) database was used to ensure the sensitivity of the searches. 40 papers met the criteria and were used for this review.
Results. Cajal cells lie in close proximity to the muscle cells, autonomic nerve endings, and urothelial cells. There is increasing evidence that ICCs play role in urinary tract dysfunction development (e.g. detrusor overactivity, primary obstructive megaureter, congenital ureteropelvic junction obstruction, etc.). ICCs may be responsible for generating electrical potentials and induction of detrusor muscle contractions. Novel pathomechanisms of detrusor overactivity development have been postulated, as follows: 1) the disturbance of spontaneous contractility caused by altered signal transduction of ICCs between nerves and detrusor muscle cells, and 2). the alteration in signal transduction between urothelium and afferent nerve endings via suburothelial ICCs. The c–kit receptor is not only a detection marker of these cells, but may also play a crucial role in the control of bladder function.
Conclusions. Cajal cells in urinary bladder suggest that the c–kit receptor may provide a novel target for treating detrusor overactivity. The review presents the current knowledge of ICCs, its role in urinary bladder function, and potential novel therapeutic strategy.

INTRODUCTION

The treatment of urge and mixed urinary incontinence is a challenging task. Particularly, as the second condition is still difficult to treat, the dilemma between surgery and conservative management is not yet resolved [1]. The discovery of the origin of bladder muscle overactivity would help patients who are suffering from an overactive bladder and its relating conditions. Past decades have shown increasing appreciation for the important role of interstitial cells of Cajal (ICCs) in gastrointestinal tract motility. The frequency of contractions and the contraction of the small bowel and the colon is the result of the local activity started in the membranes of muscle cells generated by the ICCs. ICCs are not solely responsible for, but are believed to contribute to the motility of the gut. Cajal cells are located mainly in the boundary layer of the circular and longitudinal muscle (intramuscularis, ICC–IM), but their concentrations are also observed within the Meissner and Auerbach's plexuses, andalso within deep muscular plexuses [2].

Many experimental and clinical studies have reported loss and/or damage of ICC networks leading to serious gastrointestinal dysmotility, as well as described the crucial role of ICCs in motility disorders (e.g. ulcerative colitis, Hirschprung disease, achalasia, infantile pyloric stenosis, and slow–transit constipation, etc.) in humans [3]. The pathophysiology of interstitial cells of Cajal in the regulation of the gastrointestinal tract function has been well described. These cells are specialized cells that possess self–excitability and act as primary pacemakers which inject depolarizing currents into neighbouring smooth muscles cells to initiate spontaneous slow waves and corresponding phasic contractions [2]. Moreover, ICCs seem to play a fundamental role in the transmission of signals from the enteric nervous system to the smooth muscle cells [4]. ICCs express the proto–oncogene c–kit, that encodes the tyrosine kinase receptor, Kit which is expressed by ICCs, but not smooth muscle cells or fibroblasts. The c–kit receptor is used as an identification marker of ICCs [5].

MATERIALS AND METHODS

This review is based on a systemic literature research. The medline/pubmed, scopus, embase, and Web of Science databases were browsed in order to identify original and review articles, as well as editorials relating to cajal–like cells, urinary bladder, detrusor overactivity, overactive bladder, glivec, etc. The controlled vocabulary of the Medical Subject Headings (MeSH) database was used to ensure the sensitivity of the searches. 40 papers met the criteria and were used for this review.

RESULTS

Distribution of ICCs

In the urinary bladder the interstitial cells of Cajal (ICCs) were firstly identified by Smet et al. [6]. These cells are located within the lamina propria (ICC–LP) and in the detrusor muscle (ICC – IM) of the urinary bladder with distinctive cells shapes and morphological features [7]. The ICC–LP have been identified in the lamina propria area between the urothelium and the muscularis of the detrusor and have a stellate–shaped morphology with several branches emanating from a central soma [8]. The presence of a layer of cells with the ultrastructural characteristics of myofibroblasts, being similiar, but distinct from the classical ICCs, as well as in close contact between ICC–LP and autonomic nerve endings has been described by Wiseman et al. [9] using electron microscopy. Moreover, Cajal cells always form connections with neighboring ICCs to create an interconnected cellular network that appears to be connected by connexin–43 gap junctions. Some animal studies revealed that the morphology and the distribution of ICCs is diffrent from the ICC–LP. Two sub–types of c–Kit–positive cells in the detrusor muscle can be distinguished, as follows: 1) elongated cells with several lateral branches and 2) stellate cells similar to ICC–LP. The elongated detrusor ICCs are not networked to each other, but are arranged in parallel lines which are in circular, longitudinal, and oblique orientation, reminiscent of the basket–weave array of detrusor smooth muscle bundles. These have been denoted as ICC–IM (intramuscular ICC) [10]. The ICC–IM with elongated features are located on the boundary of smooth muscle bundles and track the orientation of the bundles. Additionally, ICCs of this form are found within the smooth muscle bundles. Stellate–shaped ICCs are also found within the interstitial spaces between the detrusor smooth muscle bundles, forming regions of interconnected cells. This sub–type has been denoted ICC–IB (interbundle ICC). Both sub–types of detrusor ICC make close structural connections with nerves as demonstrated by co–labeling tissues with c–Kit and neuronal antibodies [7, 8, 10, 11]. Also, Rasmussen et al. identified two main types of interstitial cells in the urinary bladder using transmission electron microscopy, such a ICC–L (interstitial cells of Cajal–like cells) and FLC (fibroblast–like cells), as well as postulated that ICC–L may be analogous to interstitial cells of Cajal in the gastrointestinal tract. ICC–L are bipolar with slender, flattened dendritic–like processes, frequently forming a branching labyrinth network. ICC–L are interconnected by close appositions, gap junctions and peg–and–socket junctions (PSJ), but no specialised contacts to smooth muscle or nerves are apparent. In the FLC, the gap junctions and PSJ are absent and intermediate filaments are rare [12]. Many studies revealed that ICCs are characterized by positive expression of connexin–43, vimentin and cGMP [6, 9, 13]. Additionally , different types of receptors (e.g. muscarinic, vanilloid, and purinergic, etc.) were described on the surface of ICCs within suburothelial area [14, 15, 16]. Avaliable data confirms that ICCs are closely associated with detrusor smooth muscles and make structural interactions with cholinergic nerve endings [8, 17, 18 19].

ICCs in physiology/pathology and clinical implications

Based on previous experience that ICCs are pacemakers in the gastrointestinal tract, the current studies were focused on ICCs in the urinary bladder,

as a potent transducers of signals between the autonomic nerve endings and detrusor muscles. A considerable progress in the morphology, distribution and physiological properties of ICCs in the urinary bladder have been observed. Thus, these morphologic results suggest a structural foundation for bladder ICCs to act as a conduit for transmitting information from nerve fibers to detrusor myocytes [20]. Anderson KE postulated that the autonomic nervous system, ICC, and gap junctions may be involved in the modulation of detrusor muscle motor activity [21]. Previous reports suggest, that two main novel pathomechanisms may be involved in detrusor overactivity development, are as follows: 1) the disturbance of spontaneous contractility caused by altered detrusor ICCs signal transduction between autonomic nerve endings and smooth muscle cells, and 2) the disturbance of signal transduction between urothelial and sensory nerves *via* suburothelial ICCs [22]. Because of positive expression of c–kit on the surface of ICCs, the c–kit is not only a detection marker of ICCs, but may also become a cornerstone in proper control of urinary bladder function. The detrusor muscle contraction and relaxation is mediated *via* muscarinic receptors (M2 and M3 subtypes) and β–adrenoreceptors. Therefore, the interference with the signal transduction of these receptors currently became a standard treatment option for patient with overactive bladder (OAB)/detrusor overactivity (DO) [23]. Besides the status of antimuscarinics and β3–adrenoreceptor agonists as the current standard of OAB/DO treatment, further researche is needed for better understanding of OAB/DO pathophysiology and for discovering other alternative therapeutic options.

Detrusor overactivity may result from the increased coupling between detrusor smooth muscle cells [24]. Increased connexin–43–mediated intercellular communications in overactive bladder rat model has been described [25, 26]. Micromotion of the urinary bladder wall, which may be attributed to spontaneous contractions of a unit of muscle bundles, has also been reported by Drake et al. [27] to be enhanced in a rat OAB model. Thus, either quantitative or qualitative changes in ICCs may account for the increased excitability in the case of OAB/DO. Besides the standard myogenic and neurogenic concept of DO development (alterations in autonomic afferent and efferent nerves), the increased signal transmission from the afferent nerve fibres to urothelium *via* Cajal cells within suburothelial layer of bladder wall during the micturition has attracted particular considerable attention. Hashitani H. [28] suggests that ICCs seem to be active under pathological condition modulating the urinary bladder function. Par-

tial bladder outlet obstruction (PBOO) may lead to urinary bladder overactivity (OAB). An increased concentration of ICCs were found within subserosal layers and their distribution was altered in the suburothelial layer in PBOO bladders in guinea–pigs [29]. Kubota et al. [29] postulated that altered distribution and/or ultrastructural feature changes of ICCs may contribute to the pathophysiology of DO/OAB. Altered ICCs signal transduction between nerves and smooth muscle cells in the detrusor smooth muscle layer may provide the disturbance of spontaneous contractility. Moreover, the disturbance of signal transduction between urothelial cells and sensory nerves *via* suburothelial ICCs may be crucial in DO development. In painful bladder syndrome, the ICCs altered distribution and phenotype transformation to a more fibroblast–like cells have been described [30]. The up–regulation of (c–kit)–positive ICCs in human urinary bladder with OAB/DO, enhanced with connexin– 43 labeling associated with IC–LP in human and rat neurogenic bladders, support the fact that distribution of ICCs is changed in urinary bladder under pathological condition [31, 32, 36].

In the literature, there is evidence of strong correlation between urinary bladder dysfunction (e.g. detrusor overactivity) and ICC cells. Imatinib mesylate (Glivec) is a well known blocker of c–kit (tyrosine kinase) receptor, therefore it is widely used to evaluate the ICCs in the gastrointestinal tract [33]. Kil et al. [34] showed that intravenous administration of Glivec leads to the occurence of this drug in most organs, including the urinary bladder. The in vitro experiments showed that Glivec inhibits evoked smooth muscle contractions and spontaneous activity in human detrusor overactivity, with less effect on normal human tissue. Moreover, urodynamic study in the guinea–pig after systemic administration of Glivec showed improved urinary bladder capacity, bladder compliance, voided volumes, frequency, and reduced contraction thresholds and sponatneous motor activity [35]. The detrusor muscle develops phasic and autonomus activity consisting of rythmical transient contractions similar to peristaltic movements of the gastrointestinal tract during urinary bladder filling [36]. Finney et al. [37] revealed that the phasic activity occurs in the absence of neural stimulation and can be modulated by activation of different receptors (e.g. muscarinic, purinergic, etc.). The physiological detrusor phasic motor activity remains unclear, however phasic contractions may play a key role in urinary bladder function, mediating urinary bladder wall tone and relaying sensory afferent information from the detrusor. Vahabi et al. [38] confirmed that (c–kit)–positive ICCs mediate phasic activity of urinary bladder in rats. They

showed that Glivec reduces the amplitude and the frequency of carbachol (muscarinic agonist)–induced phasic contractions in control and diabetic tissues in a concentration dependent manner. Furthermore, Kubota et al. [39] reported that Glivec had inhibitory effects on detrusor muscle OAB/DO in humans and guinea–pig. Their observations suggest that ICCs may genetate bursts of action potentials and contractions in detrusor smooth muscle. Min et al. [20] postulated that Glivec affects the bladder contractile response not by directly inhibiting the contraction of detrusor muscle cells, but by directly inhibiting the function of ICCs, and thus blocking the transmission of cholinergic signals from the autonomic nerve endings to the detrusor muscle under physiologic conditions. They observed that Glivec inhibited the neurostimulation–induced bladder contractile response in a dose–dependent manner. However, no effect of Glivec on acetylocholine–induced bladder contractile response in vitro was observed. Thus, the fact that bladder ICCs are in close connection with nerve fibres, and that c–kit blocker can have an affect on the neurostimulation–induced bladder contraction would suggest that bladder ICCs play an important role in the urinary bladder excitation regulation, mostly *via* the mediation on the innervation. Deng et al. [40] investigated the effects of Glivec on the urinary bladder in rats with suprasacral cord injury (SSCI) and sacral cord injury (SCI). The results showed that urinary bladder capacity and compliance were decreased in SSCI animals, and increased in SCI rats. The amplitude and frequency of spontaneous contractions of detrusor strips, the frequency and relative fluorescence intensity of the spontaneous Ca^{2+} waves, and the c–kit expression in the bladder were significantly increased in the SSCI group and decreased in the SCI group, compared with the control and sham groups. The dose–dependent effects of Glivec also confirmed consistent functional variations in bladder activity. This experiment indicates potential roles of ICCs for the c–kit signaling pathway in the pathogenesis of urinary bladder dysfunction in SSCI and SCI animals.

McCloskey KD [32] suggests that the alteration in Cajal cells as a primary cause of urinary bladder dysfuncton is unlikely. These cells may develop alternative signaling pathways underpinning increased sensation or modulating detrusor motor activity. Moreover, improper voiding (urinary bladder motor activity) due to hypertrophy and/or fibrosis which leads to urinary bladder remodelling in many bladder diseases may explain the need for increased Cajal cells within the bladder wall to excite the hypertrophied detrusor muscle. The increased population of Cajal cells may proliferate and differentiate to myofibroblasts to supply increased extracellular matrix. The Cajal cells microenvironment alterations due to histological remodelling (fibrosis, hypertrophy or denervation) may lead to changes in their distribution due to differential availability of trophic factors [32].

CONCLUSIONS

The current knowledge of Cajal cells in the urinary bladder has raised the chances that these cells and c–kit receptors may become an interesting therapeutic target in the development of treatment strategies for an overactive bladder. Further experiments are strongly needed to answer the key question of whether the alteration of the Cajal cell population is secondary to the structural and functional changes in nerves and/or detrusor smooth muscle or whether Cajal cell changes are an early background for OAB/DO development.

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