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Different distribution of c-kit positive interstitial cells of Cajal-like in children's urinary bladders

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Abstract: We describe the presence of c-kit positive interstitial cells of Cajal-like (ICCs-like) in the walls of the urinary bladders of children. An immunohistochemical study of specimens, obtained at autopsy from either the trigonum (Group A) or the corpus (Group B), was performed using antibodies against c-kit (CD 117). Histological morphometry of the immunoexpression of c-kit positive ICCs-like was performed by means of image analysis system. The c-kit positive ICCs-like were identified by their morphology and counted in the vesical muscle layer in ten adjacent high power fields, each of 0.0479 mm^2. The areas of the epithelial and subepithelial layers containing c-kit positive mast cells (rounded body with no dendritic processes) were neglected. The results were expressed as the number of ICCs-like cells per mm^2. Differences between groups were tested using unpaired Student's *t*-test preceded by evaluation of normality and Levene's test. Results were considered statistically significant if $p < 0.05$. In Group A, the mean number of ICCs-like cells was statistically significantly higher (41.5 cells/mm^2) than in Group B (30.4 cells/mm^2), $p < 0.05$. ICCs-like cells were found within the smooth muscle layer of the urinary bladder. There was a different distribution of these cells in particular parts of the bladder, which was probably due to the different roles of the trigonum and the corpus in the bladders of children. (*Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 3, pp. 431–435*)

Key words: interstitial cells of Cajal, CD 117, immunohistochemistry, urinary bladder

Introduction

The interstitial cells of Cajal (ICCs) were first described by the Spanish neuro-histologist Ramon y Cajal in 1893. These cells are found throughout the gastrointestinal tract from the esophagus to the anus [1]. Immunohistochemistry using a c-kit antibody provides a simple and reliable way of determining the structure and distribution of ICCs. These cells play an important physiological role in the gastrointestinal tract, including the generation of electrical slow-wave activity, the facilitation of active propagation of electrical phenomena and the mediation of neurotransmission between enteric nerves and smooth muscle cells (SMCs) [2, 3].

In recent years, the presence of ICCs, ICC-like cells, interstitial cells (ICs) or myofibroblasts (there is hardly any agreement on the nomenclature) has been also identified in animal and human tissues of the urinary tract including the ureter [4–9], urinary bladder [10–14] and urethra [15, 16].

The aim of our study was to examine the distribution of c-kit positive interstitial cells of Cajal-like (ICCs-like) in the different parts of the urinary bladder's wall in children.

Material and methods

Full-thickness specimens were obtained from the urinary bladders (Group A — from the trigonum of the bladder; Group B — from the anterior wall of the corpus of the bladder) of 16 children during autopsy. The age of the children ranged from one day to ten years (Table 1). The Ethical Committee of the Polish Mothers' Health Institute in Lodz approved the study.

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Table 1. Characterization of children from Groups A and B: patient age and cause of death; mean number of ICCs-like cells per mm^2

Number of patient's specimen	Age of children at time of death	Cause of death	Group A (mean number of ICCs-like cells per mm^2) $p < 0.05$	Group B (mean number of ICCs-like cells per mm^2) $p < 0.05$
107/07	91 days	Combined heart anomaly	32.8	21.9
108/07	214 days	Combined heart anomaly	68.3	42.5
109/07	3 months	Combined heart anomaly	52.4	26.7
111/07	2 months	Combined heart anomaly	48.6	31.3
150/07	1 day	Combined heart anomaly	38.7	36.5
141/07	2 days	Combined heart anomaly	58.4	41.2
140/07	14 days	Combined heart anomaly	62.6	53.2
120/07	1 day	Meningomyelocele/hydrocephalus	26.0	22.0
145/07	30 days	Combined heart anomaly	59.7	48.1
50/10	2 days	Hematocephalus	30.0	17.0
51/10	1 day	Combined heart anomaly. Omphalocoele	25.0	15.0
52/10	3 days	Hypoplasia pulmonum. CDH	36.0	30.0
103/10	1 month	Combined heart anomaly	57.0	40.0
107/10	1 day	Hypoplasia pulmonum	39.0	23.0
120/10	10 years	Combined heart anomaly	35.0	21.0
121/10	3 years	Combined heart anomaly	34.0	18.0
Mean value	–	–	41.5	30.4

The specimens were fixed in 10% buffered formalin and embedded in paraffin. Deparaffinized sections (4 μm) were rehydrated and heat-treated in a water bath (temperature 98.5°C). Sections were then blocked for endogenous peroxidase activity by Dual Endogenous Enzyme Block and incubated with primary polyclonal rabbit anti-c-kit antibody (dilution 1:50, c-kit Oncogene Research Products, USA) for 1 h. For detection, the EnVision System — HRP (DAKO) with DAB staining and hematoxylin counterstaining were used.

Histological morphometry of the immunoexpression of c-kit (CD117) positive ICCs was performed by means of an image analysis system consisting of a PC equipped with an optical mouse, Indeo Fast card (frame grabber, true-color, real-time), produced by Indeo (Taiwan), and color TV camera produced by Panasonic (Japan) linked to a Carl Zeiss Jenaval microscope (Germany). This system was programmed (MultiScan 8.08 software, produced by Computer Scanning Systems, Poland) to calculate the number of objects (semi-automatic function). The c-kit positive ICC-like cells were identified on the basis of their morphology (fusiform cells with dendritic processes) and estimated in the vesical muscle layer and in ten adjacent high power fields, each 0.0479 mm^2. The areas of the epithelial and subepithelial layers containing c-kit positive mast cells (rounded body with no dendritic processes) were neglected. The results were expressed as the number of ICCs cells per mm^2.

Statistical methods. Differences between groups were tested using unpaired Student's *t*-test preceded by evaluation of normality and Levene's test. Results were considered statistically significant if $p < 0.05$.

Results

Our investigation indicated that c-kit positive fusiform cells with dendritic processes and with large oval nuclei were located in the vesical muscle layers. These cells displayed the morphological characteristics of cells that had previously been defined as the ICCs, or pacemaker cells in the gut. Because the anti-c-kit is a specific marker for ICCs, we referred to the labeled cells as ICCs-like.

In the urinary bladders of children, these cells commonly occurred as separate cells. But in the specimens obtained from the trigonum of the urinary bladder (Group A), small groups of c-kit positive ICCs-like accumulated to form a mass surrounded by layers of connective tissue cells. In all specimens (Groups A and B), the ICCs-like were located at the junctional area between smooth muscles fibers (Figures 1 and 2). We could not identify these cells in the vesical mucosa, lamina propria or submucosa. C-kit positive mast cells were present in these areas. These cells were

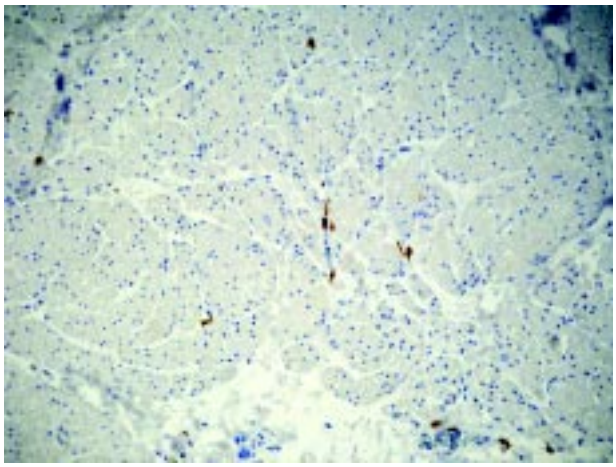

Figure 1. Distribution of c-kit positive ICCs in the muscularis propria in the corpus of a child's urinary bladder (magnification × 200)

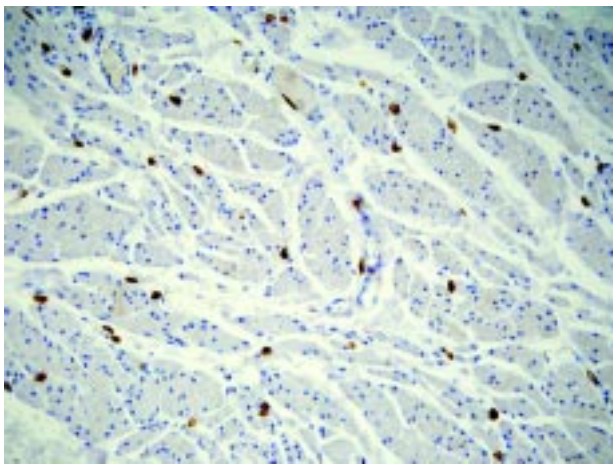

Figure 2. Distribution of c-kit positive ICCs in the muscularis propria in the trigonum of a child's urinary bladder (magnification × 200)

round and had round nuclei, but no dendritic processes as ICCs. They were sporadically present also in the muscle layer.

In Group A (specimens obtained from the trigonum of the urinary bladder) the mean value of ICCs--like was statistically significantly higher (41.5 cells/ /mm^2) than in Group B (specimens obtained from the anterior wall of the corpus of the urinary bladder) — (30.4 cells/mm^2), $p < 0.05$. Table 1 presents the mean value of ICCs-like per mm^2 for specimens obtained from the urinary bladder of each child.

Discussion

Over the last 20 years, there has been great progress in our understanding of the morphology and the physiological role of ICCs. These advances have been due to the discovery that ICCs express c-kit, the proto-oncogene that encodes the receptor tyrosine kinase, Kit. Activation of Kit signaling is required for ICCs development, differentiation and function [3, 17]. Because kit receptors are not found on the smooth muscle cells, nerves or fibroblasts, c-kit immunolabeling is a reliable method of identifying ICC-like cells in the gut and other organs [17, 18]. Morphological studies have identified different phenotypic classes of ICCs which have different roles within the gastrointestinal tract. More recently, interstitial cells with many of the morphological and electrical features of ICCs have been identified within the urinary tract of a number of mammals [7–9, 14, 16, 19–21]. As in the gastrointestinal tract, these ICCs-like cells are in most mammals immune-reactive to antibodies against the c-kit proto-oncogene.

Our current study identified c-kit positive cells in the walls of the urinary bladders of children. These cells show morphological and immunological phenotypes similar to the ICCs in the gastrointestinal tract. Our results indicate that c-kit positive ICCs are located at the junctional area between smooth muscles fibers. We could not identify these cells in the vesical mucosa, lamina propria or submucosa. Additionally, our study showed that in the specimens obtained from the trigonum of the urinary bladder, the mean number of ICCs-like cells was statistically significantly higher (41.5 cells/mm^2) than in specimens obtained from the anterior wall of the corpus of the urinary bladder (30.4 cells/mm^2), $p < 0.05$.

There are several indications in the literature that the mammalian (including the human) urinary bladder contains ICCs-like cells. The presence of interstitial cells in the guinea pig and human urinary bladders has been described using vimentin antibodies [20] or an anti-c-kit antibody [10–12]. Davidson et al. described several populations of c-kit positive ICCs-like cells throughout the guinea pig bladder wall [22]. These fall into one of three categories, namely cells in the suburothelium, cells tracing detrusor smooth muscle bundles, and cells between the bundles. Those ICCs-like cells made close contacts with nerve fibers, which suggests that they might form a communication conduit from urothelium to detrusor. Another study of guinea pigs also confirmed the location of ICCs between the muscle bundles of the detrusor from the bladder dome through the body, down to the bladder neck and into the urethra [12]. Shafik et al. identified two types of c-kit positive ICCs in the human bladder: bipolar and multipolar cells [11]. Similarly to our study, ICCs were located at the junctional area between the muscle bundles. The big mass of ICCs was detected in the superior vesical wall. The other parts of vesical wall contained ICCs in a dis-

persed fashion or collected in small groups containing a few cells [22]. The authors of this study postulated that a large collection of ICCs, detected in the dome, appeared to constitute the 'primary' vesical pacemaker that initiates the slow waves that spread to the other areas of vesical walls. They believed that a deficiency or absence of these cells may be involved in vesical motility disorders [22]. A correlation between the loss of c-kit positive ICCs from the human urinary bladder and the clinical observation in patients with megacystis-microcolon-intestinal hypoperistalsis syndrome was reported in our early study [10].

Understanding of the function of ICCs-like cells in the urinary bladder is at a comparatively early stage. While there is some evidence as to the physiological properties of the bladder ICCs from the detrusor and lamina propria regions, the precise physiological roles of ICCs in the normal bladder function have not been elucidated [6, 13, 15, 20, 21, 23]. Smet et al. [20] first described the presence of vimentin positive cells, which had the morphological appearance of ICCs, in the human and guinea pig urinary bladder, and additionally they showed that those cells responded to nitric oxide (NO) with an increase in intracellular cGMP. The study by McCloskey et al. showed that ICCs located in the guinea pig urinary bladder fire Ca^{2+} waves in response to cholinergic stimulation and can be spontaneously active [12]. This suggests that those cells could act as pacemakers or intermediates in the neuronal transmission to the smooth muscle cells. C-kit positive-ICCs have also been found in the outer detrusor muscle of the mouse bladder [13, 24]. They were closely opposed to nitric and peptergic nerve fibers, which suggested the possibility of specific innervation or interaction [13, 24]. Lagou et al. confirmed the presence of NO/cGMP sensitivity in the ICCs of the mouse urinary bladder, thus proving that ICCs generate and modulate phasic contractile activity in the outer muscle layers of the mouse bladder [24].

Conclusions

ICCs-like cells were found within the smooth muscle layers of the urinary bladder. In the specimens obtained from the trigonum of of the urinary bladder, the mean number of ICCs-like cells was higher than in the specimens obtained from the anterior wall of the corpus of the urinary bladder.

This different distribution of cells in the particular parts of the bladder probably correlates with the different roles of the trigonum and the corpus of the urinary bladder. However, additional studies are needed to delineate the functional role of ICCs in the human urinary bladder.

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