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The effects of ureteral obstruction on Cajal-like cells in rats

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Abstract *Objective:* To determine the changes in number and morphology of interstitial cells of Cajal (ICC)-like cells (ICC-LC) at the ureteropelvic junction (UPJ) of rats after experimental distal ureteral obstruction.
Materials and methods: Of a total of 109 rats, 20 served as controls (C), 20 underwent sham-operations (SH) and 69 were in the study (S) groups. The UPJs were extracted initially in the C and SH groups, and 7, 14, 30, 60 and 90 days after ligation of the distal ureter in the study groups (S1, S2, S3, S4 and S5, respectively). The sections stained by c-*kit* anticore were studied under a light microscope.
Results: The mean number of ICC-LC was 4.55 ± 2.21 in C, 5.15 ± 3.51 in SH, 7.40 ± 6.88 in S1, 21.16 ± 19.03 in S2, 12.63 ± 8.16 in S3, 10.40 ± 5.09 in S4, and 10.9 ± 6.33 in S5. There was a statistically significant increase in ICC-LC in the study groups, except S1, compared to the C and SH groups. No significant difference was detected in Cajal cell morphology and distribution pattern between groups.
Conclusions: Based on the changes in number of ICC-LC at the UPJ after obstruction of the distal ureter compared with the limited data available in the literature, we suggest that ICC-LC have a close relationship with motility of the ureter.
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Introduction

The interstitial cells of Cajal (ICC) were first described by Ramon Y. Cajal in 1893 as primitive neurons in the gastrointestinal system (GIS) [1]. Subsequent investigations revealed that these cells play a role as pacemaker cells

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Nomenclature

ICC	Interstitial cells of Cajal
c-kit	a protooncogene, activates the tyrosine kinase signal transmission pathway

between neurons and smooth muscle cells, are originally derived from the mesenchymal tissue, and are responsible for conduction of the slow wave electrical potential for peristaltic movements [2]. ICC can be detected by c-*kit*, which is a proto-oncogene that activates the tyrosine kinase signal transmission pathway [3]. A decrease in c-*kit* immunoreactivity and changes in the configuration of ICC have been reported in various motility disorders involving the GIS, such as Hirschsprung's disease, infantile hypertrophic pyloric stenosis, and slow transmission constipation [4,5].

Recently, ICC were reported at the human ureteropelvic junction (UPJ), the submucosal and muscular layers of the rat vas deferens, between the stroma of smooth muscle layers and glandular layers of guinea pig prostate, between the smooth muscle fibers and neurons of guinea pig bladder, and in the rabbit urethra [6—10]. These cells have been shown to be responsible for initiating, coordinating and producing ureteropelvic peristaltic movements at the intercaliceal area, providing for the passage of urine from the caliceal system through the ureter to the bladder [6].

Although numerous studies have been performed to describe the functions and response of ICC, the role of ICC in the urogenital system is still unclear. In this study, we evaluated the population of ICC-like cells (ICC-LC) at the rats UPJ after experimental ureteral obstruction. Since obstruction effects motility and ICC have been shown to be involved in peristaltic motility, the response of the ICC to obstruction might help us to understand better the function of ICC-LC and progression in the presence and absence of ICC-LC in the urogenital system. Further, these experimental changes may elucidate congenital and acquired motility disorders of the ureter.

Materials and methods

One hundred and nine female Wistar rats, weighing 150—250 g, and 3—4 months of age, were used. All procedures were performed at the Çukurova University Medical Sciences Experimental Research and Application Center (MSERAC) under supervision of a veterinary surgeon, according to the conditions of the 1986 Strassburg Animal Rights Universal Declaration.

The rats were separated into three major groups as follows: 20 served as controls (C), 20 underwent sham-operations (SH), and 69 were in the study (S) groups. The S groups were divided into subgroups as follows: S1,15; S2,18; S3,11; S4,15, and S5,10.

The ureters of the rats in the S groups were tied with 3-zero silk ligature approximately 3 mm proximal to the ureterovesical junction (UVJ). The SH group of rats underwent the same operation, except that the ligatures were not tied. All rats in the S groups underwent nephroureterectomy and the specimens for examination were taken from the UPJ 7, 14, 30, 60, and 90 days after the initial operation.

Ureteral ligation

The operations were performed under general anesthesia provided by 2% xylazine hydrochloride (6 mg/kg) and ketamine hydrochloride (75 mg/kg) intraperitoneally. In the S and SH groups, a 3-cm midline suprapubic skin incision was made, the fascia was reflected, the peritoneum was opened, the intestines were retracted laterally for better exposure, and the bladder was identified. The left distal ureter was dissected by a smooth hemostat clamp and tied with 3-zero sterile silk approximately 3 mm proximal to the UVJ. In the SH groups, the distal ureters were only dissected to the UVJ, but not ligated. The midline incision was closed in two layers with 3-zero polypropylene suture.

Sacrifice and removal of the ureter

The rats were sacrificed by cervical dislocation under general anesthesia on postoperative day 7 in S1, 14 in S2, 30 in S3, 60 in S4, and 90 in S5. Through a 5-cm midline suprapubic incision, the abdominal layers were opened; the left ureter and renal pelvis were dissected with a smooth hemostat clamp, and extirpated. In the C group, the rats were sacrificed by cervical dislocation under general anesthesia, and through a 5-cm midline suprapubic incision, the abdominal layers were opened, the left ureter and renal pelvis were dissected with a smooth hemostat clamp, and the UPJs were extirpated. The specimens were stored in flasks with formaldehyde stabilization at $+4\,^{\circ}C$ temperature. Histopathologic examinations were carried out on the UPJ sections of the ureter specimens, which had been cut transversely. The specimens were stained with c-*kit* anticore and toluidine blue in the Çukurova University Pathology Department Laboratories.

Histopathologic examination

Paraffin sections, 5 μm thick, were incubated at 60 °C until the paraffin melted, dewaxed using xylene, and washed with distilled water. The sections were washed in three different phosphate buffer saline (PBS) solutions (pH 7.2—7.4) for 3 min each. Avoiding air drying, 3% H_2O_2 was dripped onto the sections for 5 min at room temperature, and incubated in a 6 M urea solution on the medium mode of a microwave incubator at 95 °C for 10 min, then kept in the urea solution for 30—40 min at room temperature. The sections were rewashed in PBS and incubated with a 1:10 diluted c-*kit* polyclonal antibody (c-*kit* Ab-1 polyclonal rabbit IgG Cat # PC 34 oncogene, 100 μg, Lot # D04579-6) for 2 h and incubated with biotinylated link (anti-mouse and anti-rabbit Ig, Code No-K0672) for 20 min. The tissue pieces were subsequently incubated with streptavidin HRP (for the LSAB 2 system HRP; Dako) for 30 min following washing in PBS, and the excess solution around the tissues was removed by wiping and avoiding air drying. AEC substrate (Chromogen Ready-to-use 8, Code No-K0672) was dripped onto the specimens at room temperature for 5—15 min. The sections were rinsed with tap water after maturation was observed by microscopic examination, and counterstained with Mayer's hematoxylin for 1—2 min, then rinsed with tap water. Strict attention to avoid air-drying by working in a moist environment was maintained. The sections with Dako Ready-to-Use

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(Faramount, Aqueous, Code No-S3025) mounting medium were ready for microscopic examination.

Mucosal mast cells also have a positive c-*kit* staining pattern, and were distinguished from ICC by toluidine staining. Toluidine blue was prepared with 0.5 g toluidine and 100 cc distilled water (pH 3). Paraffin sections were dewaxed, immersed in toluidine blue solution, rinsed with tap water following an incubation period of 15 min, and immersed in xylene for 5 min after air-drying.

After coating with ethanol, the sections were studied under a light microscope (×100, 200 and 400) by two different pathologists, who were blinded to the study. The number and morphology of the submucosal ICC-LC were evaluated. The parameters in the S groups were compared with the C and SH groups. The 10 areas with the highest number of ICC-LC were identified at low power (×100), and then the number of ICC-LC in the selected fields were counted at a higher power (×200). The number of ICC-LC was recorded as the total number in 10 different areas at high power (×200).

Statistical analyses of data were performed with the Statistical Package for Social Sciences (SPSS) for Windows 11.00 using non-parametric tests. Differences among the groups were analyzed using the Kruskal–Wallis test. *P*-values < 0.05 were considered to be statistically significant.

Results

Macroscopically, ureterohydronephrosis was observed in all the rats after the obstruction was established. Microscopically, submucosal mononuclear inflammatory cells were observed in most cases. ICC-LC were identified based on brown coloration because of the AEC chromogen stain (Fig. 1). The anti-c-*kit*-stained cells contained radial branching processes. The sections were also stained with toluidine blue for differential diagnosis from mast cells, which should have stained with anti-c-*kit* as did the ICC-LC. The mast cells were ovoid/spherical shaped and had a blue–violet color with a metachromatic granular cytoplasm.

The mean number of ICC-LC was 4.55 ± 2.21 in the C group, 5.15 ± 3.51 in the SH group, 7.40 ± 6.88 in S1, 21.16 ± 19.03 in S2, 12.63 ± 8.16 in S3, 10.40 ± 5.09 in S4, and 10.9 ± 6.33 in S5 (Fig. 2, Table 1). Statistical comparisons between subgroups are presented in Table 2.

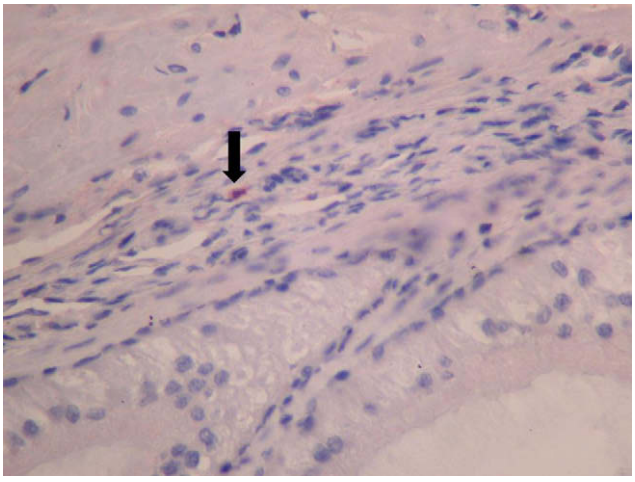

Figure 1 Anti-c-*kit*: submucosal c-*kit* + ICC on postoperative day 7 after obstruction. Reduced from ×200 (black arrow).

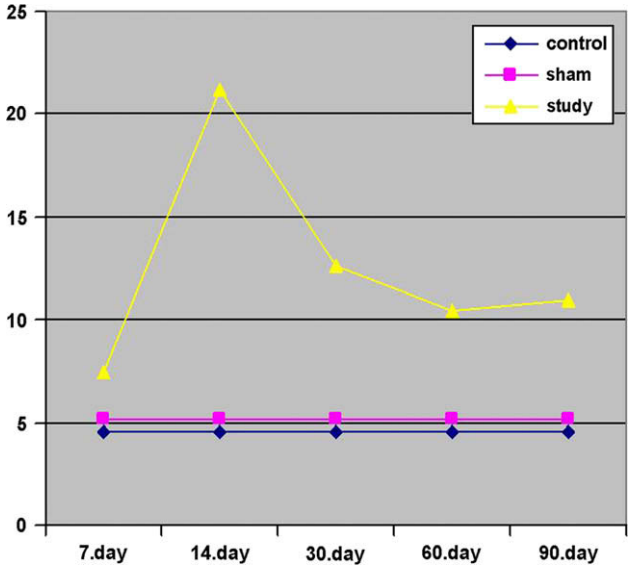

Figure 2 Graph of the results: mean number of ICC-LC over time in the three main groups.

There was no statistically significant difference in the number of ICC-LC between the C and SH groups ($P = 0.7760$). The increase in mean number of ICC-LC in all the S groups was statistically significant, except in the case of S1, when compared to the C and SH groups ($P < 0.05$). Although there was an increase in the mean number of ICC-LC in S1, this was not statistically significant ($P = 0.3204$ and $P = 0.4640$, respectively). When the S groups were compared with each other, the increase in the number of ICC-LC in groups S2, S3, S4 and S5 was significantly higher than the increase in group S1 ($P < 0.05$). There was no significant difference between groups S2, S3, S4 and S5 when compared with each other ($P > 0.05$).

Discussion

Recently, the localization, number, function and role of ICC in the urinary tract have been increasingly investigated. Initially in 1999, Klemm et al. [11] demonstrated ICC-LC in the upper urinary tract of guinea pigs morphologically and electrophysiologically. Burton et al. [12] demonstrated P_2X2 receptors of ICC in rat, mouse and pig vas deferens. Then in 2002, Exintaris et al. [8] reported the presence of the c-*kit*-immunoreactive cells between the smooth muscle

Table 1 Mean number of ICC-LC in all groups.

	Group	*n*	Mean	SD	Min.	Max.
C	Control	20	4.5500	2.21181	1.00	10.00
S	Sham	20	5.1500	3.5135	1.00	17.00
S1	7-day	15	7.4000	6.88477	1.00	22.00
S2	14-day	18	21.1667	19.03943	3.00	71.00
S3	3-day	11	12.6364	8.16422	3.00	25.00
S4	60-day	15	10.4000	5.09622	3.00	18.00
S5	90-day	10	10.9000	6.33246	1.00	19.00

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Table 2 Comparison of control, sham-operated and study groups according to number of ICC-LC.

Groups	*P*-value
C × Sham	0.77
C × S1	0.32
C × S2	0.00*
C × S3	0.00*
C × S4	0.00*
C × S5	0.00*
Sham × S1	0.46
Sham × S2	0.00*
Sham × S3	0.00*
Sham × S4	0.00*
Sham × S5	0.00*
S1 × S2	0.00*
S1 × S3	0.01*
S1 × S4	0.01*
S1 × S5	0.03*
S2 × S3	0.44
S2 × S4	0.22
S2 × S5	0.28
S3 × S4	0.74
S3 × S5	0.77
S4 × S5	0.99

*Statistically significant difference ($P < 0.05$).

stromal and glandular layers in guinea prostate, their close relationship between prostate smooth muscle and neuron fibers, the possibility of acting as pacemakers of spontaneous electrical activity, and the responsibility for the transport of glandular secretions from acinar cells to major and minor ducts of the prostate and urethra. In 2002, McCloskey and Gurney [9] described ICC in guinea pig bladder, lying parallel to smooth muscle fibers and closely related to neurons, and playing a role in conduction of signals from neurons to smooth muscle cells by firing Ca^{+2} waves spontaneously or in response to cholinergic stimuli. Sergeant et al. [10] reported interstitial cells in the rabbit urethra that were thought to have pacemaker activity and play a role in urinary continence.

In our study, we found increased numbers of ICC-LC in the submucosal layer of the UPJ 7—90 days after distal ureteral obstruction. Although the increase on the 7th day was not statistically significant, there was a tendency to increase afterwards. The peak value of ICC-LC occurred in S2 on the 14th day of the study, indicating an increase in the number of ICC-LC in the early phases of obstruction. This initial increase can be explained by an increase in precursors, and a response to probable increased peristaltic activity after obstruction in order to provide pacemaker activity. Then, a decline was detected, and the level reached a plateau towards the end of the study on the 60th and 90th days (Fig. 2). This parabole indicates a decrease in the increased proportion of ICC-LC in the late stages of the obstruction, although the decrease in these groups (S3, S4, and S5) was not significant compared to S2. This decrease might be explained by a decrease in peristaltic activity. However, the slightly increased numbers of ICC-LC in the later stages might be explained by a prolonged neuromodulator role of ICC-LC in chronic obstruction.

Solari et al. [6] reported large numbers of c-*kit*-positive ICC at the human UPJ, morphologically similar to ICC in GIS, which were responsible for initiating, coordinating and producing ureteropelvic peristaltic movements at the intercaliceal area that provides the linkage of urine from the caliceal system through the ureter to the bladder. Also, they detected that c-*kit*-positive ICC were sparse or absent in congenitally obstructed UPJ specimens. Similarly, we detected a basal number of c-*kit*-positive ICC-LC at the UPJ of rats in our C and SH groups. We also detected a significant increase in the number of ICC-LC in the submucosal layer of the ipsilateral UPJ from day 14 after unilateral distal ureteral obstruction. This situation seems to conflict with the Solari study [6], but might be explained by the difference in start times and localization of the obstructions. In Solari's study [6], the obstruction was congenital and localized to the UPJ, with decreased peristaltic activity, and might be due to congenital lack of ICC-LC. But in our study, the experimental obstruction was formed at the UVJ with increased proximal peristaltic activity. The results are similar: decreased peristaltic activity associated with decreased or absent ICC-LC and increased peristaltic activity associated with increased ICC-LC.

It is thought that pyeloureteric autorhythmicity arises in specialized electrically active pacemaker cells in the proximal regions of the upper urinary tract which drive adjacent quiescent areas. For the last 35 years, the most likely candidates for these pacemaker cells have been atypical smooth muscle cells (SMC) which have many of the morphologic and electrical characteristics of cardiac sinoatrial cells. Consistent with the presence of a proximal pelvi-calyceal pacemaker drive, atypical SMC predominate within the most proximal calyceal regions of the upper urinary tract and the number decreases with distance from the renal fornix; they have not been observed in the ureter. However, there is considerable evidence that ureters disconnected from the renal pelvis of both humans and pigs are spontaneously active *in vitro* and that rudimentary ureteric peristaltic waves can spontaneously occur *in vivo* relatively soon after kidney transplantation, pyeloureteric/ureteric surgery or obstruction [13—15].

Lang and Klemm [16] reported that pyeloureteric peristalsis depends on two populations of pacemaker cells which display fundamentally different spontaneous electrical activities: atypical SMC fire high-frequency oscillations of the membrane potential, while ICC-LC fire low-frequency action potentials. In the absence of the proximal pacemaker drive from atypical SMC after pyeloureteral/ureteral obstruction or surgery, ICC-LC pacemaking provides a compensatory mechanism allowing the ureter to maintain rudimentary peristaltic waves and movement of urine from the pyelon to the bladder [16]. This study seems to support our results. Our highest numbers of ICC-LC on the 14th day after ureteral obstruction might be due to the maintenance of rudimentary peristaltic activity, as considered in the previous study. In our study, there was a decrease in the increased proportion of ICC-LC at the late stages as the peristaltic activity decreased; the previous study also supports this situation.

Metzger et al. [17] reported that ICC-LC widely occur in the urinary tract of pigs and considered that the ubiquitous distribution of these cells and their close relationship to smooth muscle provides strong evidence that ICC-LC could

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contribute to the intrinsic pacemaker activity within the porcine upper and lower urinary tracts. This study also supports our findings.

Turunc et al. [7] demonstrated the changes in number of ICC in submucosal and muscular layers of rat vas deferens after obstruction. They found increased counts of ICC-LC 1 month after vas deferens ligation, but decreased counts after 3 months. Taken together with the results of our study, the response of ICC-LC to obstruction in both of these tubular organs seems to be similar.

In some motility disorders of the GI tract, morphologic alterations have been observed in the cells under study [4,5]. We did not determine any alterations in distribution pattern or any morphologic difference in the structure of ICC-LC in UPJ after ureteral obstruction under the light microscope. We cannot comment on the ultrastructure of ICC-LC without using transmission electron microscopy.

Conclusions

ICC-LC are good candidates for further research in a variety of important congenital and acquired urologic diseases. When the changes in number of ICC-LC at the UPJ after distal ureteral obstruction in our study are compared with the limited data available in the literature, it is possible to suggest that ICC-LC have a close relationship with ureteral motility. These cells might play a critical role in the peristaltic activity of the ureter and urine transport, especially in the presence of any ureteral disorder or surgery.

Conflict of interest statement

None.

Ethical approval

All procedures were performed at the Çukurova University Medical Sciences Experimental Research and Application Center (MSERAC) under supervision of a veterinary surgeon, according to the conditions of the 1986 Strassburg Animal Rights Universal Declaration.

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