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Original Article

Intramuscular nerve distribution in bladder and the relationship between intramuscular ganglia and bladder function in man and dog

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Abstract: In clinical, the relationship between bladder intramuscular nerve and function is also elusive. This study aims to compare the bladder intramuscular nerve distribution and its characteristics and significance in human and dog. Eleven dogs' bladders were stained by Sihler's and HE techniques. Fifteen human bladders were adopted by Sihler's staining, using 10% formaldehyde to fix 12 weeks, 7 by HE dyeing fixes 24 hours. Results indicated that man's bladder was triangularpyramid-shaped. While dog's bladder was spherical-shaped and its muscle fibers arrange were irregularly shaped. Longitudinal muscle of the outer layer is fleshy, the terminal is at the bladder neck without exception, and vesical trigone has relatively obvious three layers of structure. After dyeing dog's bladder was transparent jelly, the nerve was purple color, enter bladder at the ureter-bladder junction with different forms. Man's bladder nerves, no ganglion, were more trivial than that of dogs, and with smaller branches, the large nerve ganglion. The links with the nerve fibers and forms the network on the dog's bladder wall, and the nerve fibers crosses comparatively little on both the left and right sides in the midline. The right nerve branch gains advantage on the man's bladder wall, the situations is opposite on the dog's. In conclusion, bladder nerves which scatter to the bladder wall have branches to lower ureter at the ureter-bladder junction, the structure and distribution of intramuscular nerves are different, the existence of intramuscular ganglia is relating to the bladder function both in man and dog.

Keywords: Intramuscular nerve, bladder function, relationship, intramuscular ganglion

Introduction

Since investigation of the overall innervation of tissues and organs is critical for neural anatomy and physiology, the amount of reports on the intramuscular nerves of skeletal muscle is gradually increasing in recent years [1, 2]. Except that studies on the nerves in human thyroid have been documented, there are only a limited number of reports in other organs. Hence our study aimed to explore the human bladder intramuscular nerve distribution on the basis of canine intramuscular ganglion [3, 4] and found that human bladder walls lack of ganglia. In addition, we further compared the possible relations of the nerve distribution between the human bladders and canine bladders. This study provided detailed hints on the pathogenesis of neurogenic bladder diseases and laid the theoretical foundation for the treatment of these pathological conditions.

Materials and methods

Specimens

Eleven dog's bladder were obtained from lab after experimental surgery in our hospital, 9 fresh man's bladder, 6 corpse bladder, which all legally collected, deal with the samples with 10% formaldehyde to fix.

HE technique

Staining of 4 bladder which adopted 10% formaldehyde fixed for 24-hour canine, 3 fresh human lesions (triangle-zone and bladder tissue) which vesicoureteral junction was totally excised, with the size of 1 cm^3, after rinsing , embedded in layers and slices (from outer to inner), wax, staining, observed under the microscope scope. In addition, the excised Sihler's staining samples which the nerve fibers supply the bladder, nerve bifurcation point of intersection and black spots, observed the pictures.

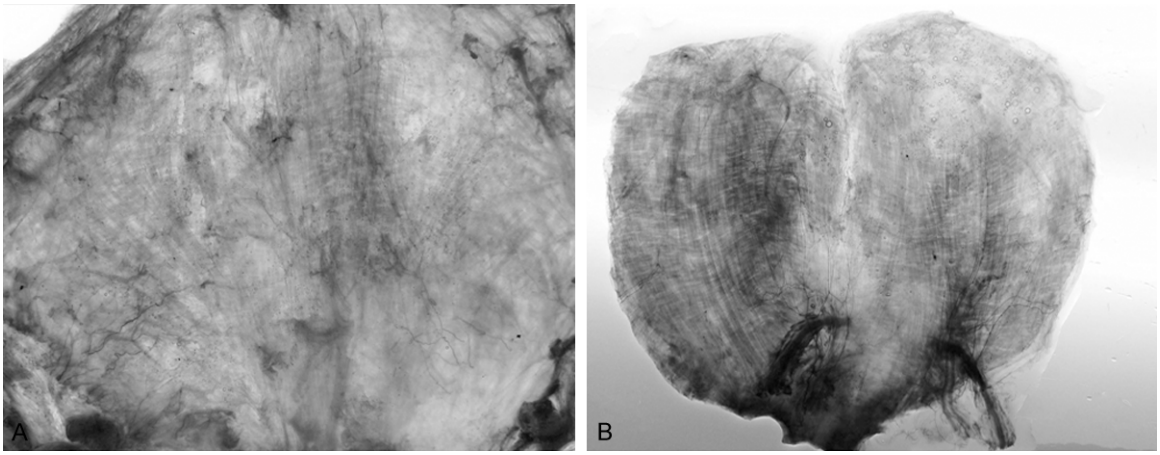

Figure 1. Comparison of bladder intramuscular innervation between human and dogs (dorsal view). A. Bladder intramuscular innervation in human; B. Bladder intramuscular innervation in dogs.

Sihler's staining

Seven dog's bladders,12 man's bladders, fixed more than 12 weeks, washed by current water for 12 hours, dissolved by 2000ml 3~1% potassium hydroxide and 0.03% hydrogen peroxide solution mixture 3~5 weeks, then Sihler's I liquid demineralized 3 weeks, Sihler's II liquid dyed 3 weeks, when the nerve was purple, nerve branches black in the Sihler's I solution, and put in 0.5 g/L lithium carbonate neutralize for 1 hour, glycerol gradient (400~1000 g/L) made the samples clear, put them on the X-ray observed and film-reading.

Results

Empty man's bladder, triangularpyramid-shaped. Dog's bladder, spherical-shaped with it's muscle fibers arrange were irregularly shaped, it's smooth muscle fibers of the muscle bundle was weaving and the thickness varied considerably. It's distribution was uncertain, and vesical trigone has a relatively clear three-layers structure. Man bladder's annular muscle of middle level is thewy, longitudinal muscle of the outer layer is fleshy (**Figure 1A**). In dog, bladder can be divided into neck, bottom, body, conus, the blood vessels, and nerves near the bladder is difficult to identify. The dog bladder with it's shape integral after Sihler's staining, muscle fibres are transparent or translucent jelly and woven, some fibres are light blue, muscle and intramuscular nerves near the neural stem branch is purple blue (**Figure 1B**). Man's bladder nerves, no ganglion, is more trivial than dog's, and with smaller branches; it's nerves distribute accompanying the inferior vesical artery, palisade-shaped at the ureter-bladder junction, some fibres envelope the ureter into the bladder wall. Dog's bladder nerves, formed the thick nerve bundle distribute and accompanying the bladder artery, primary branches envelope the ureter then supply bladder wall, it's intramuscular nerves are mainly distribute in the bladder side of the wall, along the vertical axis to the top of the extension, like dendrimers (**Figure 2**). The primary, secondary and tertiary nerve branches, scattered in different levels. The nerve fibres crosses comparatively little on both the left and right sides in the midline, some nerve fibres scatters on the layer of ureter in man's bladder, the right nerve branch gains advantage but the situations is opposite on the dog's. The intramuscular nerve fibers are visible in deep color, just as a "black spot", near the ureterovesical junction, and in the outer longitudinal muscle the "black spots" are more and larger, each "black spot" has at least two nerve fibers and they contact at different levels in dog's bladder muscle. The intramuscular nerve fibers are rich both in man and dog. The fibres on the jamb wall, triangle-zone and the middle-outer layer are denser, but they are scarce at the top and inner layer, typical of the ganglion and nerve fibers were seen in all layers' sections (**Figure 2**). In more, some ganglions are visible and have two nerve fibres and linked together, the inner ganglions have several degrees of neurons; no neurons are observed on the wall of man's (**Figure 3**). Nerve fibers of different degree are found and distrib-

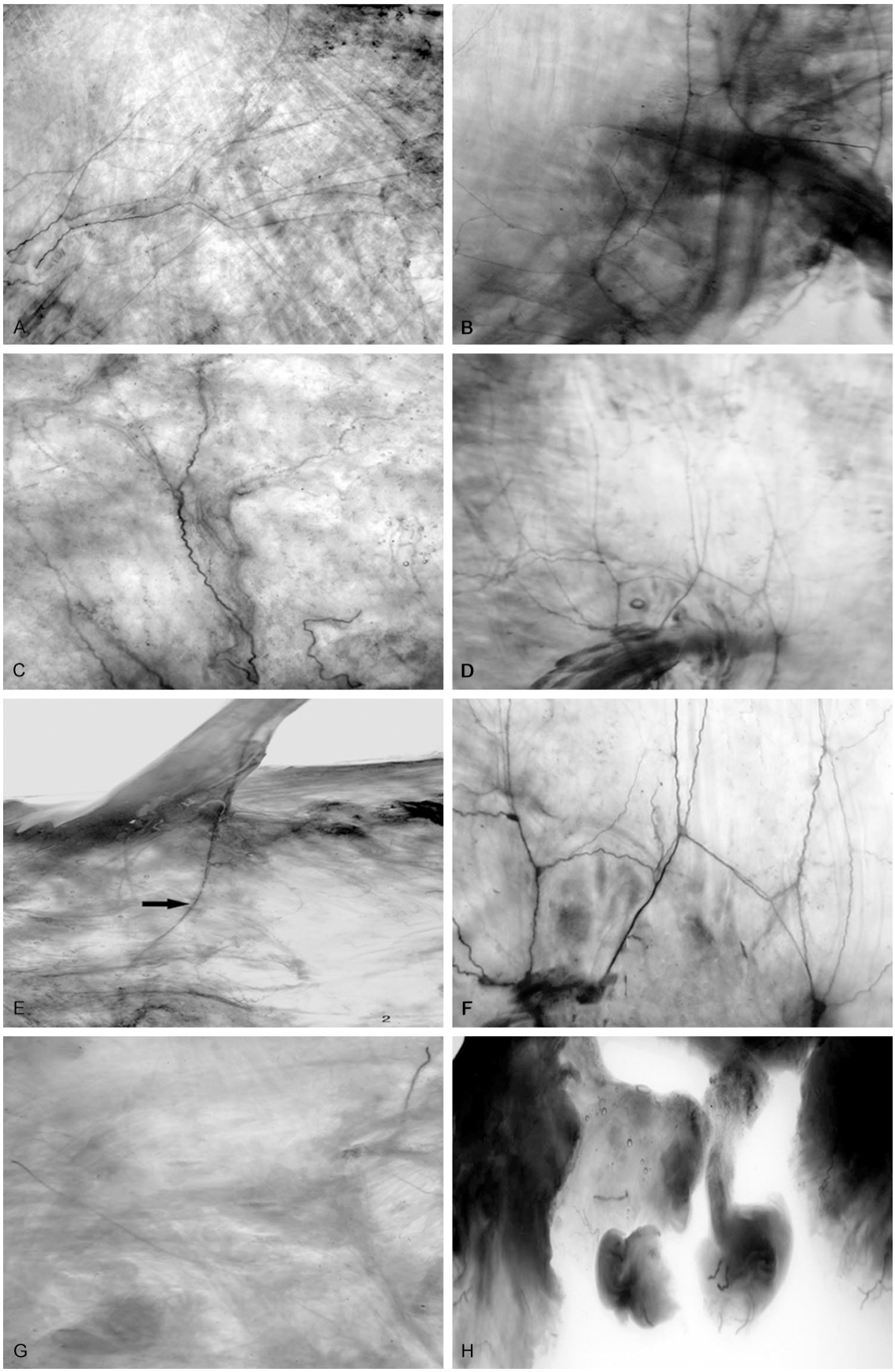
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Figure 2. Comparison of bladder intramuscular innervation between human and dogs after Sihler's staining (dorsal view). A: The bladder right intra-semimuscular nerve fiber in human; B: The bladder right intra-semimuscular nerve fiber and ganglia in dogs; C: The bladder left intra-semimuscular nerve fiber in human; D: The bladder left intra-semimuscular nerve fiber and ganglia in dogs; E: The nerve branch of lower ureter in human; F: The bladder intramuscular ganglia and nerve fibers in dogs; G: The nerve fibers of bladder triangle area and neck area in human; H: Each layer of bladder triangle area and neck area in human was innervated.

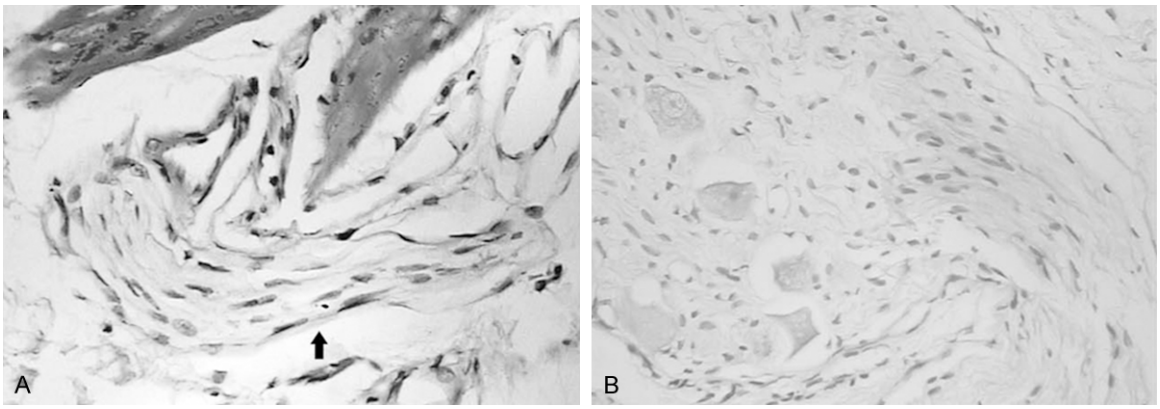

Figure 3. The bladder intramuscular nerve fibers and ganglia after HE staining in human and dogs. A: The bladder intramuscular nerve fibers (× 400). B: The bladder intramuscular nerve fibers and ganalia in dogs (× 400).

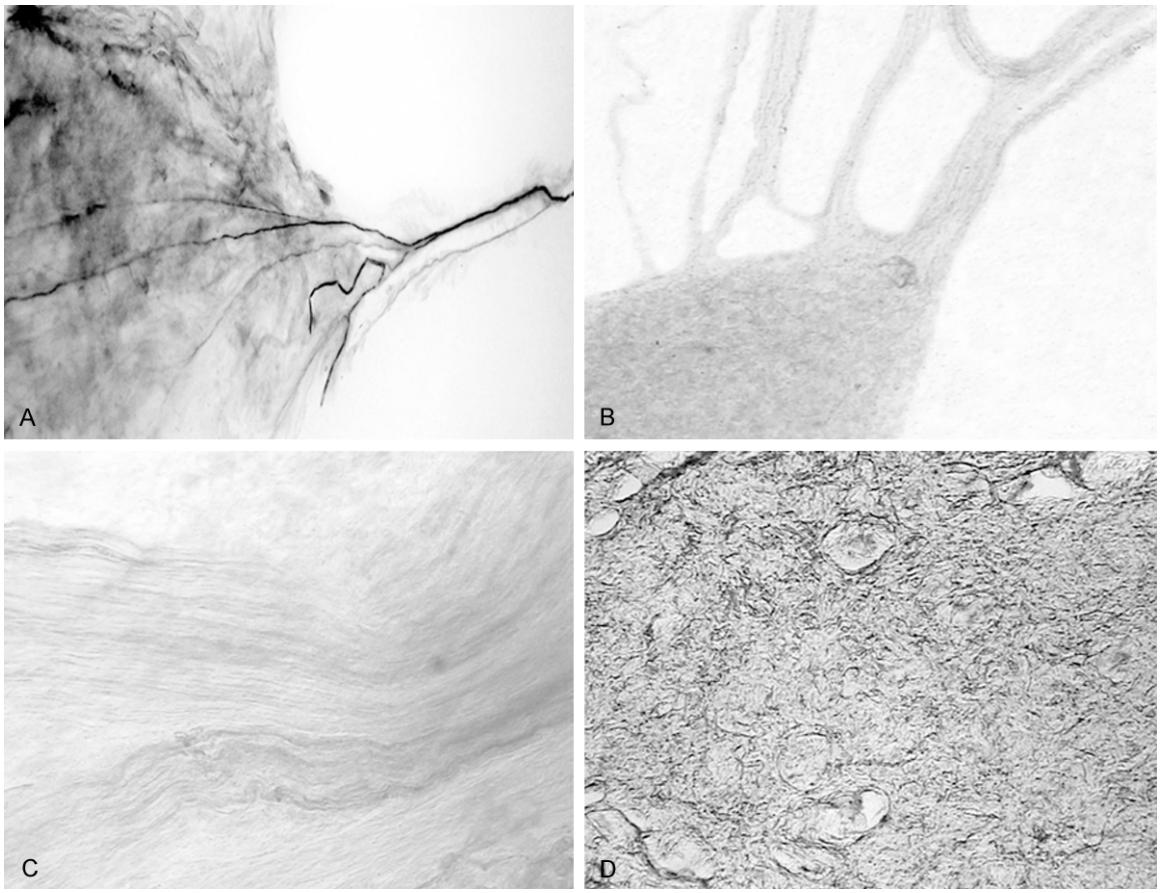

Figure 4. The observation of the bladder intramuscular nerve fibers and ganglia in human and dogs after Sihler's staining. A: The bladder intramuscular nerve fibers in human (× 100); B: The bladder intramuscular nerve fibers and

ganglia in dogs (× 100); C: The bladder intramuscular nerve fibers in human (× 100); D: The bladder intramuscular ganglia cells (× 100).

ute on different layers, the branches of adjacent nerve fibers with the fibres between, cross (**Figure 4**). Dogs' nerve fibers at the bifurcation, as the "black spots", same as the ganglion, section of the existence of neurons, but the cell structure is not clear (**Figure 4**). Each ganglion contact a number of nerve fibers, some fibers scatter separately near the ganglion, some pass through the ganglion but do not find the significant synaptic structure, the ganglion are thick and dense near vesicoureteral junction, but the similar structures were not found in man's.

Discussion

The basic functions of the urinary bladder, storage and excretion of urine mainly depend on the neuromodulation of the sympathetic and parasympathetic nerves. The sympathetic nerve are mainly located at the bladder neck, with the parasympathetic ones distributed in the bladder body [5], and such kind of distribution suits the functional regulation. Due to the different degree of evolution, the idea whether the bladder structure, function and intramural nerves distribution of man and dog are different remained still ambiguous.

Through this research, we found that empty man's bladder, triangular pyramid- shaped, its middle level of annular muscle is thewy, while dog's bladder, spherical- shaped its muscle fibers arrange were irregularly shaped, longitudinal muscle of the outer layer is fleshy. The nerves near the bladder are difficult to identify with naked eye. After Sihler's staining the shape of dog bladder is integral, muscle fibres are transparent or translucent jelly and woven. Man's bladder nerves have no ganglion, and is more trivial than dog's, also with smaller branches; its nerves distribute accompanying the inferior vesical artery, palisade-shaped at the ureter-bladder junction, some fibres envelope the ureter into the bladder wall. Whether the differences of thickness of the nerve and the nerve distribution are related to the bladder function is still unclear. Dog's bladder nerves, where the thick nerve bundles are formed distribute and accompany the bladder artery, primary branches envelope the ureter then supply bladder wall, its intramuscular nerves mainly distribute in the bladder side of the wall, along with the vertical axis to the top of the extension, like dendrimers. The primary, secondary and tertiary nerve branches, scattered in different levels. The ratio of nerve fibers and muscle fibers is not 1:1, this structure may be related to the contraction of the bladder and the ball shape changes slowly and continuously. The nerve fibres crosses comparatively little on both the left and right sides in the midline, some nerve fibres scatter on the layer of ureter in man's bladder, the right nerve branch gains advantage but the situations is opposite on the dog's, may be related to the evolution of the brain [6]. There are nerve branches to the bladder wall that also supply the lower ureter, suggesting that innervation of the ureter may be related to a similar segmental distribution of blood vessels, and interprets reasonably that the disease of lower ureter can stimulate the bladder irritation. The intramuscular nerve fibers are visible in deep blue color, which is termed the "black spot", near the ureterovesical junction, and in the outer longitudinal muscle the "black spots" are more and larger, each "black spot" has at least two nerve fibers and they contact at different levels in dog's bladder muscle. "Black spots" and its nerve fibers constitute the neural network within on the bladder wall, excise the "black spots" and their associated nerve branch, found that "black spots" is the ganglion and the structure of cells is not clear that may be caused by the stained damage, each neuron has a number of nerve fibers. Some fibers scatter separately near the ganglion, some pass through the ganglion but we do not find the significant synaptic structure, the ganglion is thick and dense near vesicoureteral junction. Fresh bladder is further stained by HE technique. The intramuscular nerve fibers are rich both in man and dog, and the fibres on the jamb wall, triangle-zone and the middle-outer layer are more dense but layer are scarce at the top and inner, typical ganglion and nerve fibers were seen in all layers' sections [7]. Moreover, some ganglions are visible and have two nerve fibres and linked together, the inner ganglions have several degrees of neurons; no similar structure is observed in man. Gabella et al. [8] reported that the nerve ganglion and fibres in

the female guinea pig bladder wall are observed by immunohistochemistry sections. BT Biallosterski [9] also found the ganglia and neurons in bladder in mice of transgenic Alzheimer disease mode. However normal mice have no nerve section, suggesting that during the process of biological evolution, the degree of evolution of the brain, the function, number and the distribution of peripheral nerve section retained some connection. Dog's brain is underdeveloped. Except for the function of storage and excrete of urine, bladder also participates in activities in urine scent marks. When the scope of their activities was beyond a certain limit, the gap will be recorded urination, less every time, short, with a pulse, but no drip pouring. Show a high degree of bladder control and loss of contractile force of principle, not "off or on" effect, which in theory requires ganglion in systolic and diastolic areas, dog bladder intramuscular ganglion may be related to the presence of precise activity [10]. Study also found the relations among the intramuscular ganglia of human bladder, whether the degree of human brain development, central functional, simplified bladder function, and degeneration of intramural ganglia are not clear. However, according to the evolutionary view, human well-developed central nervous system, the low functional central and peripheral nerve section which should be degraded, otherwise it does not match with the evolution of a highly developed nervous system that is the "information highway". The presence of intramural ganglion in part of the organ during embryonic development does not rule out the possibility of incomplete degradation. In addition, through our research, track intramuscular nerve branch to the dog bladder, we easily find the relationship between the ganglion and the nerve fiber, just like structure similar to the sympathetic chain. But whether dominance in the region is related to the fine-adjustment need further study.

Nerve crossover, anastomosis in the muscle are related to the fine regulation of muscle activity. Similar to the collateral blood supply [11, 12], it is conducive to nerve regeneration after injury and control. The mesh vertical distribution of intramuscular nerves in bladder, make it coincide with the theory that relaxant and contract, repair the damage, the intramuscular ganglion may be related to the existence of functional activities of the specific region; the place which the nerves enter into the muscle as a watching point to the ureter, can reduce the possibility of damage during the surgical process, and provides support for the neurophysiological study of bladder.

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Disclosure of conflict of interest

None.

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