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INTERSTITIAL CYSTITIS: TOWARDS BETTER UNDERSTANDING OF ITS PATHOPHYSIOLOGY.
INFLUENCE OF ALPHA1-ADRENERGIC BLOCKADE ON EXPERIMENTALLY INDUCED CYSTITIS IN FEMALE PIGS.

Hypothesis / aims of study
Almost all publications concerning interstitial cystitis (IC) begin with the phrase: “Interstitial cystitis etiopathogenesis is still far from being understood”. This lack in our knowledge is due to several factors, the main being the lack of a good and realiable animal model of this disease. IC is believed to be a specific neurogenic inflammation of the bladder. If it would be so, the selective blockade of nervous conduction should at least ameliorate interstitial cystitis produced changes. Therefore, in the present studies we wanted to validate the new animal model of an interstitial cystitis and to examine the influence of alpha1-adrenergic blockade on innervation pattern in animals with experimentally induced cystitis.

Study design, materials and methods
Experiments were performed on sexually immmature female pigs. 16 animals, divided into 4 equal groups were studied: control, control animals receiving doxazosin, animals with experimentally induced bladder inflammation and animals with induced cystitis that received doxazosin. Cystitis was achieved by combining acute bladder overdistension (pressure 100 cmH_2O) and 30 min chemical irritation with 50% acetone solution (7 days after overdistension). Doxazosin was administered orally once a day in a dose of 0.1 mg/kg for 30 days. Afterwards, animals were sacrificed, transcardially perfused and bladder scraps from the trigone and ventral bladder wall were dissected out and then processed for double immunofluorescence. Furthermore, in order to compare changes found in experimentally induced cystitis with that observed in humans with IC n=5 female patients suffering from this disease were included in the study. IC was diagnosed accordingly to the criteria of the National Institute of Arthritis, Diabetes and Digestive and Kidney Disease. The study protocol was approved by the Local EC Board. During the cystoscopy, cold-cup bladder biopsies from the posterior bladder wall were collected. Control tissue samples (n=7) were obtained during cystectomy performed for invasive transitional cell carcinoma. Tissue pieces were collected from unchanged region of the posterior bladder wall and then processed for double-immunofluorescence. Distribution pattern of the following antigens were studied in nerve fibers supplying human and porcine bladder wall, using the following: tyrosine hydroxylase (TH; mouse monoclonal, 1:60) or dopamine-β-hydroxylase (DβH; mouse monoclonal, 1:400 or rabbit polyclonal, 1:4000; as markers for sympathetic nerve fibres), substance P (SP; rat monoclonal, 1:350), calcitonin gene-related peptide (CGRP; rabbit polyclonal, 1:4000), serotonin (5-HT; rabbit polyclonal, 1:2000), pituitary adenylate cyclase-activating polypeptide (PACAP; rabbit polyclonal, 1:4000), neuropeptide Y (NPY; rat monoclonal, 1:350 or rabbit polyclonal, 1:4000) and vasoactive intestinal polypeptide (VIP; mouse monoclonal, 1:800 or rabbit polyclonal, 1:4000). The antigen-antiserum complexes visualized with appropriate secondary antisera.

Results
Mean patients age was 63.8 $\pm$ 6.3 years, mean disease duration was 3.3 $\pm$ 2.1 years. Neoplasmatic cells were not observed in specimens of human urinary bladder studied by means of routinely performed histopathological examination. In patients with IC an increase in the expression of CGRP-immunoreactive (IR), PACAP-, NPY- and VIP-IR nerve fibers was observed, while the number of fibers being SP-IR remained unchanged. Similar findings were observed in specimen from experimental animals, however no increase in PACAP- and VIP-IR nerve fibers was observed. Details are presented in Tab. 1.

Table I. Semi-quantitative evaluation of the expression pattern of various neuropeptides within the suburothelial layer of the urinary bladder.

Substance	Human		Pig	
	Control	IC	Control	Cystitis
SP	+	+	+	+
CGRP	+	++	+	++
VIP	++	+++	++	++
PACAP-27	+	++	+	+
DβH	++	++	++	++
5-HT	+	-	+	-
NPY	++	+++	++	+++

Arbitrary evaluation of the relative density of studied nerve terminals: - no nerve fibers; + few fibers; +: moderate number of fibers; +++ numerous nerve terminals.

Furthermore, we observed a dramatic increase in CGRP-IR nerve fibers in control animals following doxazosin administration, however no such changes were observed in inflamed bladders. A similar situation was observed for DβH-IR terminals, except the fact that in inflamed bladder doxazosin produced almost complete depletion of DβH-IR nerve terminals. 5-HT-IR within the submucosa of inflamed bladder was returning to its original density (as observed in control animals), a similar observation was made for NPY-IR and VIP-IR fibers. In the case of VIP-IR fibers, however, these changes were restricted to the muscular layer. No clear-cut changes were observed in the case of PACAP- and SP-IR terminals within the bladder wall.

Interpretation of results

Our findings demonstrate that the population of CGRP and SP-IR nerve fibers underwent the same changes in the human IC and experimentally induced cystitis in pigs. This makes the proposed model an interesting option for study of interstitial cystitis pathogenesis. Moreover, it appears that doxazosin is able to interfere both with the sensory and autonomic component of the bladder innervation. However, in the case of experimentally induced bladder inflammation the most pronounced changes were observed within the sympathetic part of the nerve fibers subpopulation. This could be the manifestation of “salvage” reflex development.

Concluding message

This study corroborates the view that porcine bladder innervation resembles in many respects that of the human organ and that the changes in the sensory innervation of experimentally inflamed porcine bladder are very similar to human organ with IC. Furthermore, doxazosin appears to influence both sympathetic and sensory nervous systems of the bladder in experimentally induced cystitis.

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